

FORM PTO-1399 (Modified) (REV 10/95)		U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE		ATTORNEY'S DOCKET NUMBER 21373.003	
TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371				U.S. APPLICATION NO. (IF KNOWN, SEE 37 CFR <b>097446373</b> )	
INTERNATIONAL APPLICATION NO. <b>PCT/DE98/01689</b>		INTERNATIONAL FILING DATE <b>19 June 1998</b>		PRIORITY DATE CLAIMED <b>20 June 1997</b>	
TITLE OF INVENTION <b>OXIDATION AND BLEACHING SYSTEM WITH ENZYMATICALLY PRODUCED OXIDIZING AGENTS</b>					
APPLICANT(S) FOR DO/EO/US <b>Call, Hans-Peter</b>					
Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:					
<ol style="list-style-type: none"> <li>1. <input checked="" type="checkbox"/> This is a <b>FIRST</b> submission of items concerning a filing under 35 U.S.C. 371.</li> <li>2. <input type="checkbox"/> This is a <b>SECOND</b> or <b>SUBSEQUENT</b> submission of items concerning a filing under 35 U.S.C. 371.</li> <li>3. <input checked="" type="checkbox"/> This is an express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).</li> <li>4. <input checked="" type="checkbox"/> A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.</li> <li>5. <input checked="" type="checkbox"/> A copy of the International Application as filed (35 U.S.C. 371 (c) (2))             <ol style="list-style-type: none"> <li>a. <input checked="" type="checkbox"/> is transmitted herewith (required only if not transmitted by the International Bureau).</li> <li>b. <input checked="" type="checkbox"/> has been transmitted by the International Bureau.</li> <li>c. <input type="checkbox"/> is not required, as the application was filed in the United States Receiving Office (RO/US).</li> </ol> </li> <li>6. <input type="checkbox"/> A translation of the International Application into English (35 U.S.C. 371(c)(2)).</li> <li>7. <input type="checkbox"/> A copy of the International Search Report (PCT/ISA/210).</li> <li>8. <input type="checkbox"/> Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371 (c)(3))             <ol style="list-style-type: none"> <li>a. <input type="checkbox"/> are transmitted herewith (required only if not transmitted by the International Bureau).</li> <li>b. <input type="checkbox"/> have been transmitted by the International Bureau.</li> <li>c. <input type="checkbox"/> have not been made; however, the time limit for making such amendments has NOT expired.</li> <li>d. <input type="checkbox"/> have not been made and will not be made.</li> </ol> </li> <li>9. <input type="checkbox"/> A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).</li> <li>10. <input checked="" type="checkbox"/> An oath or declaration of the inventor(s) (35 U.S.C. 371 (c)(4)).</li> <li>11. <input type="checkbox"/> A copy of the International Preliminary Examination Report (PCT/IPEA/409).</li> <li>12. <input type="checkbox"/> A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371 (c)(5)).</li> </ol>					
Items 13 to 18 below concern document(s) or information included:					
<ol style="list-style-type: none"> <li>13. <input type="checkbox"/> An Information Disclosure Statement under 37 CFR 1.97 and 1.98.</li> <li>14. <input type="checkbox"/> An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.</li> <li>15. <input type="checkbox"/> A <b>FIRST</b> preliminary amendment.</li> <li>16. <input type="checkbox"/> A <b>SECOND</b> or <b>SUBSEQUENT</b> preliminary amendment.</li> <li>17. <input type="checkbox"/> A substitute specification.</li> <li>18. <input type="checkbox"/> A change of power of attorney and/or address letter.</li> <li>19. <input checked="" type="checkbox"/> Certificate of Mailing by Express Mail</li> <li>20. <input checked="" type="checkbox"/> Other items or information:</li> </ol>					
<b>PCT/RO/139</b> <b>PCT/IB/308</b> <b>PCT/IB/332</b> <b>PCT/IPEA/402</b> <b>PCT/RO/101</b> <b>WO 98/59108 (Intl. Publication of Application)</b> <b>Verified Statement Claiming Small Entity Status</b>					

U.S. APPLICATION NO. (IF KNOWN, SEE 37 CFR

INTERNATIONAL APPLICATION NO.

ATTORNEY'S DOCKET NUMBER

09/1446373

PCT/DE98/01689

21373.003

20. The following fees are submitted:

**BASIC NATIONAL FEE ( 37 CFR 1.492 (a) (1) - (5)) :**

- ☐ Search Report has been prepared by the EPO or JPO ..... \$840.00
- ☐ International preliminary examination fee paid to USPTO (37 CFR 1.482) ..... \$670.00
- ☐ No international preliminary examination fee paid to USPTO (37 CFR 1.482) but international search fee paid to USPTO (37 CFR 1.445(a)(2)) ..... \$760.00
- ☒ Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO ..... \$970.00
- ☐ International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(2)-(4) ..... \$96.00

**CALCULATIONS PTO USE ONLY****ENTER APPROPRIATE BASIC FEE AMOUNT =**

\$970.00

Surcharge of \$130.00 for furnishing the oath or declaration later than months from the earliest claimed priority date (37 CFR 1.492 (e)). ☐ 20 ☐ 30

\$0.00

CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE	
Total claims	- 20 =	0	x \$18.00	\$0.00
Independent claims	- 3 =	0	x \$78.00	\$0.00
Multiple Dependent Claims (check if applicable).			<input type="checkbox"/>	\$0.00

**TOTAL OF ABOVE CALCULATIONS =**

\$970.00

Reduction of 1/2 for filing by small entity, if applicable. Verified Small Entity Statement must also be filed (Note 37 CFR 1.9, 1.27, 1.28) (check if applicable).

☒

\$485.00

**SUBTOTAL =**

\$485.00

Processing fee of \$130.00 for furnishing the English translation later than months from the earliest claimed priority date (37 CFR 1.492 (f)). ☐ 20 ☐ 30

\$0.00

**TOTAL NATIONAL FEE =**

\$485.00

Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31) (check if applicable).

☐

\$0.00

**TOTAL FEES ENCLOSED =**

\$485.00

Amount to be:	\$
refunded	
charged	\$

- ☒ A check in the amount of \$485.00 to cover the above fees is enclosed.
- ☐ Please charge my Deposit Account No. in the amount of to cover the above fees.  
A duplicate copy of this sheet is enclosed.
- ☒ The Commissioner is hereby authorized to charge any fees which may be required, or credit any overpayment to Deposit Account No. 18-1215 A duplicate copy of this sheet is enclosed.

**NOTE:** Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

Susan S. Jackson  
Kennedy Covington Lobdell & Hickman, LLP  
4200 Bank of America Corporate Center  
100 North Tryon Street  
Charlotte, NC 28202-4006  
Telephone: (704) 331-7400  
Facsimile: (704) 331-7598

SIGNATURE

Susan S. Jackson

NAME

41,302

REGISTRATION NUMBER

December 20, 1999

DATE

**VERIFIED STATEMENT (DECLARATION) CLAIMING SMALL ENTITY  
STATUS (37 CFR 1.9(f) AND 1.27 (b)) - INDEPENDENT INVENTOR**

Docket No.

Serial No.  
To Be AssignedFiling Date  
Concurrently HerewithPatent No.  
PCT/DE98/ 01689

Issue Date

Applicant/  
Patentee: Hans-Peter Coll

Invention:

Oxidation and bleaching system with enzymatically produced oxidizing agents

As a below named inventor, I hereby declare that I qualify as an independent inventor as defined in 37 CFR 1.9(c) for purposes of paying reduced fees under section 41(a) and (b) of Title 35, United States Code, to the Patent and Trademark Office with regard to the invention entitled above and described in:

- ☒ the specification to be filed herewith.  
☐ the application identified above.  
☐ the patent identified above.

I have not assigned, granted, conveyed or licensed and am under no obligation under contract or law to assign, grant, convey or license, any rights in the invention to any person who could not be classified as an independent inventor under 37 CFR 1.9(c) if that person had made the invention, or to any concern which would not qualify as a small business concern under 37 CFR 1.9(d) or a nonprofit organization under 37 CFR 1.9(e).

Each person, concern or organization to which I have assigned, granted, conveyed, or licensed or am under an obligation under contract or law to assign, grant, convey, or license any rights in the invention is listed below:

- ☒ No such person, concern or organization exists.  
☐ Each such person, concern or organization is listed below.

\*NOTE: Separate verified statements are required from each named person, concern or organization having rights to the invention averring to their status as small entities (37 CFR 1.27)

FULL NAME  
ADDRESS☐ Individual☐ Small Business Concern☐ Nonprofit OrganizationFULL NAME  
ADDRESS☐ Individual☐ Small Business Concern☐ Nonprofit OrganizationFULL NAME  
ADDRESS☐ Individual☐ Small Business Concern☐ Nonprofit OrganizationFULL NAME  
ADDRESS☐ Individual☐ Small Business Concern☐ Nonprofit Organization

I acknowledge the duty to file, in this application or patent, notification of any change in status resulting in loss of entitlement to small entity status prior to paying, or at the time of paying, the earliest of the issue fee or any maintenance fee due after the date on which status as a small entity is no longer appropriate. (37 CFR 1.28(b))

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this verified statement is directed.

NAME OF INVENTOR Hans-Peter CallSIGNATURE OF INVENTOR Hans-Peter CallDATE: 17. December 1999

NAME OF INVENTOR \_\_\_\_\_

SIGNATURE OF INVENTOR \_\_\_\_\_

DATE: \_\_\_\_\_

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DATE: \_\_\_\_\_

09/446373

WO98/59108

PCT/DE 98/01689

# OXIDATION AND BLEACHING SYSTEM WITH ENZYMATICALLY PRODUCED OXIDIZING AGENTS

It is known from a number of literature references and review articles, for example from "Preparative Biotransformations" by S.M. Roberts, K. Wiggins and G. Casy, J. Wiley & Sons Ltd, that enzymes such as certain lipases are capable of forming epoxides via the formation of peroxy acids (per fatty acids). For example, in the lipase system (from *Candida antarctica*), with continuous addition of  $H_2O_2$  and in the presence of certain fatty acids, for example tetradecanoic (myristic) acid or dodecanoic (lauric) acid, from cyclooctene the corresponding epoxide is produced. It is also known that manganese peroxidases + unsaturated fatty acids can generate peracids which in turn can act as a  $H_2O_2$  source for manganese peroxidases (literature: B.W. Bogan et al., Applied and Environmental Microbiology, vol. 62, No. 5, pp. 1788-1792).

There are also a few patents that describe the formation of peracids with the aid of haloperoxidases.

It is also known that dimethyldioxirane can be generated in situ from peracids or salts of peracids (such as Oxone) and acetone as the simplest ketone. Ketones other than acetone are also used. Dioxiranes can also be prepared as pure substances before they are used as oxidants, but their stability is problematical (WO 92/13993). Moreover, it is known from Canadian Patent 1 129 162, US 5, 034 096 and WO 96/13634 that certain metal ions, for example  $Mo^{6+} + H_2O_2$  and nitrilamides +  $H_2O_2$  and dicyandiamides +  $H_2O_2$  are capable of generating dioxiranes chemically from  $H_2O_2$ . In this case, surprisingly, certain combinations are possible with the enzyme components system (ECS) of the present invention (see below), namely the enzymatic generation of activated oxygen species e.g. dioxiranes can be further enhanced.

These very strong and highly selective oxidants can be used in many oxidation reactions (for example epoxide reactions etc). Recently, it has been proposed to use them, in particular, as bleaching agents in the cellulose/pulp industry. Because of the dangerous preparation and high cost, this proposal has not found acceptance (WO 92/13993).

The object of the present invention is to provide a highly selective oxidation or bleaching system for use in cellulose/pulp bleaching or high yield wood pulp bleaching, in the oxidative treatment of wastewater of all kinds, in the preparation of wood-based composites, as an enzymatic deinking system, as an oxidant in organic synthesis, in coal liquefaction, as bleaching system in detergents and as a bleaching agent or oxidant in the textile industry (for example in stone washing and fabric bleaching). Said system does not present many of the drawbacks of purely chemical systems (for example environmental pollution problems) or of enzymatic systems (which often show inadequate performance and are very costly).

Surprisingly, we have now found, that an oxidation system which contains certain lipases, oxidants such as  $H_2O_2$ , certain fatty acids and certain ketones resulted, for example, in bleaching of cellulose/pulp while at the same time the kappa number

(delignification) was markedly reduced, i.e. it could be clearly demonstrated, surprisingly, that when the appropriate optimal components were present in an optimum proportion and concentration relative to each other, it was possible to achieve in the aforesaid cellulose/pulp bleaching a bleaching action comparable to that of dioxirane-forming chemical systems.

Surprisingly, it was also possible to demonstrate considerable bleaching action in the bleaching of high yield wood pulp, in the bleaching of pulps after a deinking processes, in oxidative polymerization of lignin and/or lignin-like substances and in the oxidative treatment of wastewater of all kinds, such as wastewater from high yield wood pulp preparation (groundwood, refiner pulp), from the cellulose/pulp industry and dye-contaminated wastewater, for example from the textile industry. For most of these wastewaters, besides the decolorization and oxidation and thus the "destruction" of environmental pollutants, the polymerization of lignins is the preferred application, because it causes a marked increase in molecular size and permits an easier and substantially less expensive precipitation of these polymers and thus their elimination from COD considerations.

Surprisingly, we were also able to demonstrate this oxidative polymerization of lignin and/or lignin-like substances in the preparation of wood-based composites (binder and/or adhesive preparation) by oxidative polymerization of the polyphenylpropanes present. Moreover, surprisingly, we were able to demonstrate removal of printing inks in the deinking process (probably occurring by swelling of the lignin-containing waste paper fibers). Surprisingly, we were also able to observe coal liquefaction properties in the treatment of lignite or anthracite. Moreover, also surprisingly, we found a marked and selective oxidation power in the use as "oxidant" in organic synthesis, high bleaching power when used as bleaching additive to detergents, in the general bleaching of textile fabrics and as special bleach when used in the stone washing processes, namely as a replacement for mechanical color removal and/or as postbleach in these processes. It seems possible, that the responsible oxidants(s), for example, generated from the present ketones and peracids are dioxiranes which in the above-indicated applications serve as oxidants or bleaching agents either alone or in combination with the peracids formed.

The foregoing objective is reached also by providing an enzyme component system (ECS) according to the invention which contains one or more lipases, preferably from the group of triacylglycerol lipases (3.1.1.3), or one or more amidases, preferably from the group of amidases (3.5.1.4) (**system component 1**) which from one or more fatty acids present (preferably C<sub>6</sub>-C<sub>26</sub> and particularly C<sub>8</sub> - C<sub>16</sub> fatty acids) (**system component 2**) and in the presence of an oxidant such as a peroxide, preferably H<sub>2</sub>O<sub>2</sub> (**system component 3**) can produce peracids and in the presence of ketones as additional component (**system component 4**), for example, dioxiranes.

Description of Various Applications of the Enzyme Component System (ECS) of the Invention

**I) Use in the bleaching of cellulose/ wood pulp.**

Use:

- a) in the treatment of, primarily, wood pulp wastewater in the pulp and paper industries, and
- b) of wastewater in other industries.

**III) Use in the preparation of lignin solutions or gels, of the corresponding binders/adhesives and of wood-based composites.**

**IV) Use as enzymatic deinking system.**

**V) Use as oxidation systems in organic synthesis.**

**VI) Use in coal liquefaction.**

**VII) Use as bleaching agent in detergents.**

**VIII) Use in the bleaching/decolorization of textile fabrics.**

**I) Use of the Enzyme Component System (ECS) of the Invention in the Bleaching of Cellulose/Wood Pulp**

Wood pulp is currently produced mainly by the sulfate and sulfite processes. By both processes, pulp is made by cooking at high temperature and under pressure. The sulfate process involves the addition of NaOH and Na<sub>2</sub>S, whereas the sulfite process uses Ca(HSO<sub>3</sub>)<sub>2</sub>, + SO<sub>2</sub>, although the sodium and ammonium hydrogen sulfite salts are currently used because of their higher solubility.

The main objective of all processes is the removal of lignin from the plant material, wood or annual plants used.

The lignin, which together with the cellulose and hemicellulose forms the main constituent of the plant material (stalks and stems), must be removed, because it is otherwise not possible to produce nonyellowing, mechanically highly resistant papers.

The processes for making high yield wood pulp involve the use of stone grinders (groundwood) or of refiners (TMP = thermomechanical pulp) which after an appropriate pretreatment (chemical, thermal or thermechemical) defibrillate the wood by milling.

These wood pulps still contain most of the lignin. They are used primarily for the production of newspapers, magazines etc.

The possibilities of using enzymes for lignin degradation have been under investigation for several years. The mechanism of action of such lignolytic systems was discovered

only a few years ago, when it became possible to obtain sufficient amounts of enzymes from the white rotting fungus *Phanerochaete chrysosporium* by use of proper culturing

conditions and the addition of inductors. This is how the hitherto unknown lignin peroxidases and manganese peroxidases were detected. Because *Phanerochaete chrysosporium* is a very effective lignin degrader, attempts have been made to isolate its enzymes and use them in purified form for lignin degradation. This was unsuccessful, however, because it was found that the enzymes primarily cause repolymerization of lignin and not its degradation.

The same is true for other lignolytic enzyme species, such as the laccases which degrade lignin oxidatively with the aid of oxygen rather than hydrogen peroxide. It was found that similar processes are at work in all cases, namely that radicals are formed which then react with each other causing the mentioned polymerization.

Currently, there are only processes based on the use of in-vivo systems (fungal systems). Optimization attempts were directed mainly toward **biopulping and biobleaching**.

By biopulping is meant the treatment of wood chips with live fungal systems. There are two kinds of application forms:

1. Pretreating the wood chips before charging them to the refiners or milling for the purpose of saving energy in high yield wood pulp production (for example, TMP or groundwood). Another advantage is the usually achieved improvement of mechanical properties of the stock, and a drawback is that the final brightness is worse.
2. Pretreating the wood chips (softwood/hardwood) before wood pulp cooking (kraft process, sulfite process). Here, the objective is to reduce the amount of digestion chemicals, to improve digestion capacity and extended cooking. The advantages include improved kappa number reduction following digestion compared to digestion without pretreatment.

The drawbacks of these processes are clearly their long treatment times (several weeks) and particularly the unsolved problem of risk of contamination during the treatment, if it is desired to omit the uneconomical sterilization of the wood chips.

Biobleaching also uses in-vivo systems. Before bleaching, the digested pulp (softwood/hardwood) is inoculated with the fungus and treated for a period of days or weeks. Only after such a long treatment time is it possible to observe a drop in kappa number and a significant improvement in brightness, so that the process is uneconomical for implementation in current bleaching sequences.

Another application, mostly carried out with immobilized fungal systems, is the treatment of pulp production wastewaters, particularly bleaching plant wastewaters, for the purpose of decolorizing them and reducing the AOX value (reducing the amount of chlorinated compounds in the wastewater, compounds which were used for chlorine or chlorine dioxide bleaching). It is also known to use hemicellulases and particularly xylanases and mannanases as bleach boosters.



These enzymes act mainly on the reprecipitated xylan, which after the cooking process partly covers the residual lignin, for the purpose of degrading it and thus improving accessibility to the lignin of the bleaching chemicals (primarily chlorine dioxide) used in the subsequent bleaching sequences. The savings in bleaching chemicals demonstrated in the laboratory have been confirmed on a large scale only to a limited extent so that this type of enzyme must also be classified as a bleaching additive.

Patent application PCT/EP 87/00635 describes a system for removing lignin from lignin-cellulose- containing material with simultaneous bleaching. The system is based on the use of lignolytic enzymes from white rotting fungi with the addition of reducing agents, oxidants and phenolic compounds as mediators.

According to DE 4 008 893 C2, in addition to the redox system, mimicking substances are added which simulate the active center (prosthetic group) of lignolytic enzymes. In this manner, a marked improvement in performance is achieved.

According to patent application PCT/EP 92/01086, additional improvement is achieved by use of a redox cascade with the aid of phenolic or nonphenolic aromatics "balanced" in terms of their oxidation potential.

All three processes are limited in regard to their applicability on an industrial scale in that they must be used at low wood pulp consistency (up to a maximum of 4%) and, in the case of the last two applications also by the risk of leaching out metals when chelating agents are used, the metals possibly causing peroxide decomposition in the subsequent peroxide bleaching stages.

WO 94/12619, WO 94/12620 and WO 94/12621 disclose processes in which the peroxidase activity is increased with enhancers. Enhancers are characterized in WO 94/12619 in terms of their half-life.

According to WO 94/12620, enhancers are characterized by the formula  $A=N-N=B$  where N means nitrogen, A and B are defined cyclic groups. According to WO 94/12620, enhancers are organic chemicals containing at least two aromatic rings of which at least one is substituted with defined groups.

All three patent applications concern dye transfer inhibition and the use of enhancers together with peroxidases as detergent additives or detergent compositions used in the detergent sector.

Although the applicability to lignin is mentioned in the specification of the applications, our own tests with the substances actually disclosed in these applications have shown that the claimed mediators are ineffective in increasing the bleaching action of peroxidases in the treatment of lignin-containing materials!

WO 94/29510 and WO 96/18770 describe a process for enzymatic delignification whereby the enzymes are used together with mediators. The mediators disclosed are, in general, compounds characterized by the structure NO-, NOH- or NRNOH.

Among the mediators disclosed in WO 94/29510 and WO 96/18770, 1-hydroxy-1H-benzotriazole (HOBT) gave the best delignification results. HOBT, however, has several drawbacks:

- it is available only at a relative high price and in insufficient quantities,
- under delignification conditions, it reacts forming 1 H-benzotriazole and other colored products, this compound shows relatively low degradability and could, in large amounts, present a pollution problem,
- to a certain degree, it harms the enzymes,
- its delignification velocity is not very high.

Other mediator of the described NO-, NOH- and HRN-OH type do not show most of these drawbacks, but still have the disadvantage that a relatively large amount of chemicals must be used and, particularly, that because of their physiological reactivity they may not be entirely harmless (mostly because of NO- radical formation).

It is therefore desirable to provide systems for modifying, degrading or bleaching lignin, lignin-containing materials or similar substances, which do not have the said drawbacks or present them only to a minor degree.

Quite surprisingly, we have now found that when the enzyme component system (ECS) of the invention is used, similar or better delignification and bleaching results are achieved compared to the abovesaid oxidoreductase-mediator systems, and the said drawbacks are negligible.

In other words, according to the invention, the foregoing objective is reached by providing an enzyme component system (ECS) according which contains one or more lipases, preferably from the group of triacylglycerol lipases (3.1.1.3), or one or more amidases, preferably from the group of amidases (3.5.1.4) (**system component 1**) which from one or more fatty acids present (preferably C<sub>6</sub>- C<sub>26</sub> and particularly C<sub>8</sub> - C<sub>16</sub> fatty acids) (**system component 2**) and in the presence of an oxidant such as a peroxide, preferably H<sub>2</sub>O<sub>2</sub> (**system component 3**) can produce peracids and in the presence of ketones as additional component (**system component 4**), for example, dioxiranes.

## **Ila) Use of the Enzyme Component System (ECS) of the Invention in the Enzymatic Treatment of Special Wastewaters (Paper Industry Wastewaters from, for Example, Groundwood Plants or Refiner Plants)**

Unlike most enzymes, oxidases and peroxidases exhibit low substrate specificity, namely they can convert a wide range of substances, usually of phenolic nature. Without mediators, oxidases as well as many peroxidases show the tendency to polymerize phenolic substances via free radical-induced polymerization, a property which is attributed, for example, to laccase, belonging to the group of oxidases, also in nature. The ability to polymerize appropriate substances, for example lignins, namely to increase the size of the molecules involved by "coupling reactions", can be utilized, for example, for the treatment of lignin- containing wastewaters in the paper industry such as TMP wastewater (wastewater from the production of thermomechanical pulp by means of refiners) and of grinder wastewater from mechanical wood pulping units.

The water-soluble lignin compounds (polyphenolpropanes) contained in these wastewaters are mainly responsible for the high COD (chemical oxygen demand) and cannot be removed by conventional technology. In the water treatment plant and in the downstream waters, they are not degraded at all or they are degraded only very slowly. At very high concentrations, these compounds can even inhibit the bacteria in a water treatment plant and thus create problems.

In this application, the enzyme action can be observed immediately by a rapid development of turbidity in the wastewater being treated, caused by an enlargement and thus insolubilization of lignin molecules. The target molecules (polymerized lignin) thus enlarged in molecular weight by enzymatic catalysis can be removed by appropriate treatments (by flocculation, by precipitation with, for example, aluminum sulfate/sodium aluminate, optionally in the presence of cationic or anionic polyelectrolytes or by sedimentation). The wastewater then shows a markedly reduced COD. Upon disposal, such wastewater causes less pollution, namely it increases the certainty of remaining below the permissible COD limits. This is particularly important for not infrequently used "procedures" run at the limit.

For this treatment, for example with laccase, the cost of removing the reaction products of the enzymatic treatment by flocculation, sedimentation or precipitation or a combination of several such methods constitutes by far the predominant part of the overall cost of the process.

We have now found, quite surprisingly, that when the enzyme component system (ECS) of the invention is used by employing a special combination of the components, much higher efficiency than be attained than with the above-described enzymatic systems. This means that the process according to the invention represents a substantially improved system compared to the aforesaid systems employing oxidoreductases (such

as, for example, laccases) as oxidation catalysts, the advantages of which are, in particular, its higher oxidation power and the use of readily degradable fatty acids and ketones (for example, benzophenones) which, although raising the COD temporarily, are readily removed in the subsequent steps carried out in the water treatment plant.

In other words, according to the invention, the foregoing objective is reached by providing an enzyme component system (**ECS**) according to which contains one or more lipases, preferably from the group of triacylglycerol lipases (3.1.1.3), or one or more amidases, preferably from the group of amidases (3.5.1.4) (**system component 1**) which from one or more fatty acids present (preferably C<sub>6</sub>-C<sub>26</sub> and particularly C<sub>8</sub>-C<sub>16</sub> fatty acids) (**system component 2**) and in the presence of an oxidant such as a peroxide, preferably H<sub>2</sub>O<sub>2</sub> (**system component 3**) can produce peracids and in the presence of ketones as additional component (**system component 4**), for example, dioxiranes.

To this system are added other special compounds (**polymerization catalysts**) which serve as condensation nuclei and can substantially enhance lignin polymerization so that the main objective of this enzymatic wastewater treatment, which is to use the lowest possible amount of the cost-intensive precipitant, can be attained.

## IIb) Use in the Enzymatic Treatment of Other Industrial Wastewaters

All other industrial wastewaters containing phenolic or, in general, oxidizable substances (for example, lignin, dyes etc) can, in principle, be treated with, for example, the abovesaid oxidoreductases. Such treatment can be applied to wastewaters from grape presses, olive presses, dyeing plants in the textile industry, wastewaters from pulping plants etc. If at all possible, to attain maximum efficiency, polluted streams should be treated before they are combined with other wastewaters.

In this case, too, we found, surprisingly, that the use of the enzyme component system (**ECS**) of the invention is very well suited for the treatment of the abovesaid wastewaters and in some cases shows performance advantages over the oxidoreductase systems. Here, too, the abovesaid special compounds, namely **polymerization catalysts**, are used. These substances consist of phenols, phenol derivatives or other polycyclic phenolic compounds with a number of oxidizable hydroxyl groups. Preferably, such **polymerization catalysts** are, for example:

alizarin, 5-amino-2-hydroxybenzoic acid, 3-aminophenol, pyrocatechol, 2,2-bis(4-hydroxyphenyl)- propane, bis(4-hydroxyphenyl)methane, quinalizarin, 4-chloro-1-naphthol, coniferyl alcohol, 2,4-di- aminophenol dihydrochloride, 3,5-dichloro-4-hydroxyaniline, 1,4-dihydroxyanthraquinone, 2,2-di- hydroxybiphenyl, 4,4-dihydroxybiphenyl, 2,3-dihydroxynaphthalene, 2,6-diisopropylphenol, 3,5-di- methoxy-4-hydroxybenzhydrazine, 2,5-ditert.butylhydroquinone, 2,6-ditert.butyl-4-

methylphenol, 4-hydroxybiphenyl, 2-hydroxydiphenylmethane, 2-(2-hydroxyphenyl)benzothiazole, 5-indanol, 2-iso-propoxyphenol, 4-isopropyl-3-methylphenol, 5-isopropyl-2-methylphenol, 4-isopropylphenol, lauryl gallate, 2-naphthol, 4-nonylphenol, 3-(pentadecyl)phenol, 2-propylphenol, 4-propylphenol, purpurine, pyrogallol, 4-(1,1,3,3-tetramethylbutyl)phenol, 1,2,4-trihydroxybenzene, 2,4,6-trimethylphenol, 2,3,5-trimethylphenol, 2,3,6-trimethylphenol, 3,4,5-trimethylphenol, 6,7-dihydroxy-4-methyl coumarin, 2-(2-hydroxyethoxy)benzaldehyde, 1-naphthol, nordihydroguaiaretic acid, octyl gallate, silibinin, 3,4,6-trihydroxybenzoate-octylester, 2,4,6-tritert butylphenol, 2,4-ditert.butylphenol, 2,6-dichlorophenol, indophenol, ethoxyquin, 1-aminoanthraquinone, 2-amino-5-chlorobenzophenone, 4-aminodi-phenylamine, 7-amino-4-hydroxy-2-naphthalenesulfonic acid, 2-(4-aminophenyl)-6-methylbenzothiazole, benzanthrone, trioctyl trimellitate, trans-chalcone, bis(4-aminophenyl)amine sulfate, 2,2'-ethylidenebis(4,6-ditert butylphenol), 2,2-bis(2,6-dibromo-4-(2-hydroxyethoxyphenyl)propane, bis(3,5-ditert butyl-4-hydroxyphenyl)methane, 2,2-bis(3,5-dichloro-4-hydroxyphenyl)propane, Bismarck Brown Y, 1-bromophthalin, 4-butylaniline, 2-tert.butyl-5-methylphenol, 1-chloro-anthraquinone, 2-chloroanthraquinone, triallyl 1,3,5-benzenetricarboxylate, 1,1,1-tris(hydroxymethyl)propane, tri-methacrylate, pentaerythrityl triacrylate, 1,2,4-trivinylcyclohexane, trans,cis-cyclododeca-1,5,9-triene, pentaerythritol tetrabenzoate, 4,4'-methylenebis(2,6-ditert.butylphenol), 4,4'-isopropylidene-bis(2,6-dichlorophenol), 4,4'-isopropylidene-bis(2,6-dibromophenol), 4,4'-isopropylidene-bis(2-(2,6-dibromophenoxy)ethanol, 2,2'-ethylidene-bis(4,6-ditert butylphenol), 3-tert.butyl-4-hydroxy-5-methylphenol, 5-tert.butyl-4-hydroxy-2-methylphenol, syringaldazine, 4,4'-dimethoxytriphenylmethane and di-sec butylphenol.

Also particularly preferred are compounds with several hydroxyl groups, such as:

ellagic acid, gallic acid, gallein, gallangin, myoinositol, morin, nitranilic acid, phenolphthalein, purpurin, purpurogallin, quinizarin, chrysazin, quercitin, quinhidrone, chloranilic acid, carmine, rhodizonie acid, croconic acid, melitic acid, hematoxylin, 9-phenyl-2,3,7-trihydroxy-6-fluorene, 9-methyl-2,3,7-trihydroxy-6-fluorene, tetrahydroxy-p-benzoquinone, 2,2',4,4'-tetrahydroxybenzophenone, Pyragallol Red, 1-nitrophloroglucinol, 1,4-dihydroxyanthraquinone, 5,8-dihydroxy-1,4-napthoquinone, hexa-oxocyclohexane octahydrate, 5,7-dihydroxyflavanone, 3',4'-dihydroxyflavanone, glyoxal hydrate, 1,3,5-tris(2-hydroxyethyl)isocyanuric acid, quinalizarin and 2,4,5-trihydroxybenzamine.

### III) Use of the Enzyme Component System of the Invention in the Preparation of Lignin Solutions or Gels, of the Corresponding Binders/Adhesives and of Wood-Based Composites

The object of the present invention is to provide a process for enzymatic polymerization and/or modification of lignin or lignin-containing materials, for example for use in the production of wood compositions or wood-based composites such as, for example, fiber board from disintegrated wood or particle board from wood chips or wood pieces (chipboard, plywood, wood composite beams).

It is known from the literature and patents, for example WO 94/01488, WO 93/23477, WO 93/25622 and DE 3 037 992 C2 that laccases, lignin peroxidases or peroxidases can be used for this purpose. The main drawbacks, particularly in the case of laccases and lignin peroxidases, are the difficulty of preparing these enzymes and the low yields even of genetically modified systems.

We have now found, quite surprisingly, that here, too, the enzyme component system (ECS) of the invention shows much better performance compared to the prior-art enzymatic systems for the polymerization and/or modification of lignin and/or lignin-containing materials.

In other words, according to the invention, the foregoing objective is reached by providing an enzyme component system (ECS) according which contains one or more lipases, preferably from the group of triacylglycerol lipases (3.1.1.3), or one or more amidases, preferably from the group of amidases (3.5.1.4) (**system component 1**) which from one or more fatty acids present (preferably C<sub>6</sub>- C<sub>26</sub> and particularly C<sub>8</sub> - C<sub>16</sub> fatty acids) (**system component 2**) and in the presence of an oxidant such as a peroxide, preferably H<sub>2</sub>O<sub>2</sub> (**system component 3**) can produce peracids and in the presence of ketones as additional component (**system component 4**), for example, dioxiranes.

To this end, the enzyme component system of the invention is brought together with lignin (for example, with lignosulfates and/or unevaporated or evaporated sulfite waste liquor and/or sulfate lignin --> kraft lignin, for example induline) and/or with lignin-containing material. The lignin and/or the lignin-containing material can either be preincubated at an elevated pH, namely above pH 8 and preferably at a pH between 9.5 and 10.5, at 20 to 100 °C (preferably at 60 to 100 °C) after which the pH is reduced to below pH 7, depending on the optimum pH range for enzyme activity of the component system (ECS) or, if the activity optimum of the enzyme component system (ECS) is on the alkaline side, the ECS and the lignin and/or the lignin-containing material are brought together immediately, without pretreatment. The purpose of the pretreatment or treatment under alkaline pH conditions is to utilize the substantially easier solubilization of lignin at these higher pH values. This is a major advantage for the use according to the invention, because it is thus possible to work without an organic solvent.

In other words, the main purpose of the described bringing together of the enzyme component system and the lignin and/or lignin-containing material is to achieve activation of the substrates (polyphenylpropanes) by oxidation, namely to convert the lignin and/or the lignin-containing material by free radical-induced polymerization (modification) into an activated and active binder which then when brought together with the wood fibers and/or wood parts to be bonded (cemented together) can be cured under the action of pressure and elevated temperature to give solid wood-based composites such as the abovesaid wood products, for example fiber boards and particle boards. The main advantage consists of reducing, or producing savings in, the amount of urea-formaldehyde resins normally used, for example, for gluing in the production of chipboard, which resins, besides being toxic, have only limited moisture resistance, or of phenol-formaldehyde resins which exhibit unfavorable swelling properties and require long pressing times (once again, besides the question of toxicity).

The polymerizing and/or modifying action of the enzyme component system can be additionally enhanced by addition of certain chemical polymerization catalysts, for example polydiphenylmethane diisocyanate (PMDI) and other polymerization catalysts

used also for the polymerization of lignin in lignin-containing wastewaters. Such substances consist of phenols, phenol derivatives or other polycyclic phenolic compounds with a number of oxidizable hydroxyl groups, as already indicated hereinabove (wastewater treatment).

#### **IV) Use of the Enzyme Component System (ECS) as an Enzymatic Deinking System**

In principle, by deinking, which is currently always run in a conventional manner by flotation, is meant a two-step process.

Its objective is to remove printing ink and other dye particles from the waste paper. The waste paper used in most cases is paper collected domestically and consists mainly of newspapers and magazines.

In the first treatment step, the dye particles adhering to the paper fibers are removed primarily by mechanical/chemical means. This is accomplished by "recycling" the paper as a uniform fibrous slurry, namely by disintegrating (comminuting) the waste paper in pulpers, drums or the like with simultaneous addition of chemicals capable of enhancing removal and preventing yellowing and thus also acting as bleaching chemicals, namely sodium hydroxide solution, fatty acid, water glass and hydrogen peroxide ( $H_2O_2$ ). Here, the fatty acid acts as a fiber dye particle collector and in the second treatment step, the flotation, also as foaming agent.

After the waste paper has been disintegrated and the said chemicals have been allowed to act for a certain length of time, the flotation is carried out in special flotation vessels by injecting air. During this process, the dye particles adhere to the foam bubbles and are removed together with the bubbles. The dye is thus separated from the paper fibers. Currently, this operation is preferably carried out at a neutral pH, which makes it necessary to use certain detergents in place of the fatty acids.

It is known from the literature (WO 91/14820, WO 92/20857) to use an oxidoreductase or laccase system characterized primarily by the addition of special substances which cause the optimum pH for the action of laccase obtained from *Trametes versicolor*, which normally is in the range of about pH 4-5, to shift into the slightly alkaline range (pH 8 to 8.7). This, on the one hand, is an important prerequisite for use in the deinking system because of the  $CaSO_4$  problems arising below pH 7 and, on the other, does not optimize the action of laccase in the polymerizing or depolymerizing sense, but only produces a certain swelling of the fibers. Such swelling (which is one of the main actions of sodium hydroxide in purely chemical deinking systems) is a primary performance characteristic of the dye-removing mechanism.

The only other additives required for this enzymatic system employing oxidoreductases are the detergents needed to produce foam. Nearly all suitable detergents also exert a dye-removing action. Moreover, in conventional deinking systems the use of sodium hydroxide and peroxide improves brightness as a result of the bleaching action of these

chemicals. This bleaching action cannot be achieved with the prior-art enzyme system because of the nature of the system.

We have now found, quite surprisingly, that by appropriate selection of the components the enzyme component system (ECS) of the invention exceeds the efficiency of other enzymatic deinking systems, particularly those with oxidoreductases and those applied to lignin-containing deinked pulp and at least in part compensates for the advantage of bleaching with purely chemical systems. This means that it is possible to provide a system offering environmentally friendly deinking under neutral pH conditions and thus better post-bleaching, better pulp properties etc and good performance similar to that of purely chemical systems.

In other words, according to the invention, the foregoing objective is reached by providing an enzyme component system (ECS) according which contains one or more lipases, preferably from the group of triacylglycerol lipases (3.1.1.3), or one or more amidases, preferably from the group of amidases (3.5.1.4) ( **system component 1**) which from one or more fatty acids present (preferably C<sub>6</sub>- C<sub>26</sub> and particularly C<sub>8</sub> – C<sub>16</sub> fatty acids) (**system component 2**) and in the presence of an oxidant such as a peroxide, preferably H<sub>2</sub>O<sub>2</sub> (**system component 3**) can produce peracids and in the presence of ketones as additional component (**system component 4**), for example, dioxiranes.

In this case, a further improvement of printing ink removal can be attained by the aforesaid addition of special substances mostly of a phenolic nature and, in particular, containing several hydroxyl groups, which are also used as polymerization catalysts in enzymatic wastewater treatment and general polymerization reactions, as in the production of binders/adhesives from lignin or lignin-containing substances primarily for the preparation of wood-based composites.

#### **V) Use of the Enzyme Component System (ECS) of the Invention as an Oxidation System in Organic Synthesis**

Recently, enzymes have increasingly been used for chemical reactions in organic synthesis. A few examples showing a variety of oxidative reactions that can be carried out with enzymatic systems can be found in: Preparative Biotransformations (Whole Cell and Isolated Enzymes in Organic Synthesis), S.M. Roberts, K. Wiggins and G. Casy, J. Wiley & Sons Ltd, 1992/93; Organic Synthesis with Oxidative Systems, H.L. Holland, VCH, 1992; and Biotransformations in Organic Chemistry, K. Faber, Springer Verlag [publisher], 1992:

##### **1) Hydroxylation reactions**

- a) Synthesis of alcohols
- b) Hydroxylation of steroids
- c) Hydroxylation of terpenes
- d) Hydroxylation of benzenes
- e) Hydroxylation of alkanes
- f) Hydroxylation of aromatic compounds
- g) Hydroxylation of double bonds
- h) Hydroxylation of nonactivated methyl groups



- i) Dihydroxylation of aromatic compounds

## 2) Oxidation of unsaturated aliphatics

- a) Preparation of epoxides
- b) Preparation of compounds by epoxidation
- c) Preparation of arene oxides
- d) Preparation of phenols
- e) Preparation of cis-dihydrodiols

## 3) Baeyer-Villiger oxidations

- a) Baeyer-Villiger conversion of steroids

## 4) Oxidation of heterocycles

- a) Transformation of organic sulfides
- b) Oxidation of sulfur compounds
- c) Oxidation of nitrogen compounds (formation of N-oxides etc.)
- d) Oxidation of other heteroatoms

## 5) Carbon-carbon dehydrogenation

- a) Dehydrogenation of steroids

## 6) Other oxidation reactions

- a) Oxidation of alcohols and aldehydes
- b) Oxidation of aromatic methyl groups to aldehydes
- c) Oxidative coupling of phenols
- c) Oxidative degradation of alkyl chains ( $\beta$ -oxidation etc.)
- e) Formation of peroxides or percompounds
- f) Initiation of free-radical induced chain reactions.

Here, too, we found, quite surprisingly, that with the aid of the enzyme component system (ECS) of the invention it is possible to carry out many oxidation reactions exemplified hereinabove, namely that the aforesaid objective can be reached by providing an enzyme component system (ECS) according to the invention which contains one or more lipases, preferably from the group of triacylglycerol lipases (3.1.1.3), or one or more amidases, preferably from the group of amidases (3.5.1.4) (**system component 1**) which from one or more fatty acids present (preferably  $C_6 - C_{26}$  and particularly  $C_8 - C_{16}$  fatty acids) (**system component 2**) and in the presence of an oxidant such as a peroxide, preferably  $H_2O_2$  (**system component 3**) can produce

peracids and in the presence of ketones as additional component (**system component 4**), for example, dioxiranes.

## VI) Use of the Enzyme Component System (ECS) in Enzymatic Coal Liquefaction

In this field, the prior art is as follows:

Preliminary studies show that, in principle, lignite and anthracite can be attacked and liquefied by in-vivo treatment with, for example, a white rotting fungus such as *Phanerochaete chrysosporium* (incubation time: several weeks/Bioengineering 8, 4, 1992). The possible structure of anthracite is a tridimensional network of polycyclic, aromatic ring systems with a "certain" similarity to lignin structures. Assumed cofactors besides the lignolytic enzymes are chelating agents (siderophors, such as ammonium oxalate) and biosurfactants.

- 1) Until now, effective coal liquefaction systems are known only as in-vivo systems (with lignin- degrading organisms, particularly white rotting fungi), or as systems employing oxidoreductases plus mediators (laccase-mediator system -> WO 94/29510; WO 96/18770).
- 2) It has been proven that, in principle, white rotting fungi that are capable of degrading lignin in vivo can also liquefy coal in culture.
- 3) Coal: Both lignite and anthracite were formed from wood by chemical/physical "actions"; hence, their chemical structures are at least similar to those occurring in lignin.
- 4) In coal liquefaction with white rotting fungi, we see, on the one hand, an alkalization of the pH during fungal growth "on coal" and, on the other, a secretion of siderophor-like chelators, namely substances known to have a positive effect on coal liquefaction.

The main reason for economical, meaningful industrial coal liquefaction is the industrial demand for alternative liquid sources of energy, especially considering that in the future the quantities of other sources of fossil energy such as oil and gas will be decreasing while at the same time the demand for energy will be increasing, and that other alternatives such as nuclear fusion, among others, will not yet be available.

Here, too, we found, quite surprisingly, that with the aid of the enzyme component system (ECS) of the invention liquefaction of, for example, lignite is possible with better performance than with the conventional enzymatic oxidoreductase systems, namely that the aforesaid objective can be reached by providing an enzyme component system (ECS) according to the invention which contains one or more lipases, preferably from the group of triacylglycerol lipases (3.1.1.3), or one or more amidases, preferably from the group of amidases (3.5.1.4) (**system component 1**) which from one or more fatty acids present (preferably C<sub>6</sub>- C<sub>26</sub> and particularly C<sub>8</sub> - C<sub>16</sub> fatty acids) (**system component 2**) and in the presence of an oxidant such as a peroxide, preferably H<sub>2</sub>O<sub>2</sub> (**system component 3**) can produce peracids and in the presence of ketones as additional component (**system component 4**), for example, dioxiranes.

## VII) Use of the Enzyme Component System (ECS) as Bleaching Agent in Detergents

Conventional bleaching systems in domestic detergents are unsatisfactory, particularly in the low- temperature range. Below a washing temperature of 60 °C, the standard bleaching agent, i.e.,  $\text{H}_2\text{O}_2$  / sodium perborate/sodium percarbonate must be activated by the addition of chemical bleach activators, such as TAED and/or SNOBS\*. Also, the need exists for more highly biodegradable, bio-compatible bleaching systems and systems for low-temperature washing that can be used in small amounts. Whereas enzymes are already being used industrially for protein-starch-fat solution and for fiber treatment in the washing process, no enzymatic system is currently available for detergent bleaching. WO 1/05839 describes the use of different oxidative enzymes (oxidases and peroxidases) to prevent dye transfer. As is known, peroxidases are capable of "decolorizing" different pigments (3-hydroxyflavone and betaine are decolorized by horseradish peroxidase and carotene by peroxidase). Said patent application describes the decolorization (also referred to as bleaching) of textile dyes removed from the laundered goods and present in the washing liquor (conversion of a colored substrate into a noncolored, oxidized substance). In this case, the enzyme is said to have the advantage over, for example, hypochlorite which attacks dyes in or on the fabric, in that the enzyme decolorizes only the dissolved dyes. Hydrogen peroxide or an appropriate precursor generating hydrogen peroxide in situ participates in the catalysis of the decolorization. The enzyme reaction can be enhanced somewhat by addition of other oxidizable enzyme substrates, for example metal ions such as  $\text{Mn}^{2+}$ , halide ions, such as  $\text{Cl}^-$  or  $\text{Br}^-$  or organic phenols, such as p-hydroxycinnamic acid and 3,4-dichlorophenol. In this case, it is postulated that short-lived radicals or other oxidized states of the added substrate are formed and are responsible for the bleaching or other modification of the colored substance.

US 4, 077 6768 describes the use of iron porphyrin, hemin chloride, iron phthalocyanines or derivatives thereof together with hydrogen peroxide for preventing dye transfer. These substances, however, are rapidly destroyed by excess peroxide, and for this reason hydrogen peroxide formation must occur in a controlled fashion. Processes are known from WO 94/12619, WO 94/12620 and WO 94/12621 whereby the activity of the peroxidase is enhanced by means of enhancers. Such enhancers are characterized in WO 94/12620 in terms of their half-life. According to WO 94/12621, enhancers are characterized by the formula  $\text{A} = \text{N}-\text{N} = \text{B}$  where N means nitrogen and A and B are defined cyclic groups. According to WO 94/12620, enhancers

\* TAED = tetraacetythylenediamine; SNOBS = sodium nonyloxybenzenesulfonate

are organic chemicals containing at least two aromatic rings of which at least one is substituted with defined groups.

All three patent applications concern dye transfer inhibition and the use of enhancers together with peroxidases as detergent additives or detergent compositions used in the detergent sector. The combination of these enhancers is limited to peroxidases. The use of mixtures containing peroxidases is also known from WO 92/18687. A special system of oxidases and of appropriate substrates such as hydrogen peroxide is described in German Unexamined Patent Application DE-42 31 761. German Unexamined Patent Application DE 19 18 729 concerns another special detergent system consisting of glucose and glucose oxidase or of starch, aminoglucosidase and glucose oxidase (GOD) and of added hydroxylamine or a hydroxylamine compound, wherein the hydroxylamine or the derivatives thereof serve to inhibit the catalase that is often present in GOD. Hydroxylamine and the derivatives thereof have definitely not been described as mediator additives.

Finally, WO 94/29425, DE 4445088.5 and WO 97/48786 concern multicomponent bleaching systems for use with detergents and which consist of oxidation catalysts and oxidants and of aliphatic, cycloaliphatic, heterocyclic or aromatic compounds containing NO-, NOH- or H-NR-OH groups.

All hitherto known "enzymatically enhanced" detergent-bleaching systems have the drawback that their cleaning and bleaching action is still unsatisfactory and that the mediators must be used in excessive amounts which may cause environmental and economic problems.

We have now found, quite surprisingly, that the enzyme component system (ECS) of the invention exceeds the performance of the aforesaid oxidoreductase-mediator systems and does not have the said drawbacks of the prior art, namely that the aforesaid objective can be reached by providing an enzyme component system (ECS) according to the invention which contains one or more lipases, preferably from the group of triacylglycerol lipases (3.1.1.3), or one or more amidases, preferably from the group of amidases (3.5.1.4) (**system component 1**) which from one or more fatty acids present (preferably C<sub>6</sub>-C<sub>26</sub> and particularly C<sub>8</sub>-C<sub>16</sub> fatty acids) (**system component 2**) and in the presence of an oxidant such as a peroxide, preferably H<sub>2</sub>O<sub>2</sub> (**system component 3**) can produce peracids and in the presence of ketones as additional component (**system component 4**), for example, dioxiranes.

#### **VIII) Use of the Enzyme Component System of the Invention in the Bleaching and/or Decolorization of Textile Fabrics**

Enzymes are currently being used to an increasing extent in various applications in the textile industry. For example, the use of amylases in the desizing process is of great importance, because the use of strong acids, alkalies or oxidants is thereby avoided. Similarly, cellulases are used for biopolishing and biostoning, a process which is mostly employed together with conventional stone washing with pumice in the treatment of denim fabrics for jeans to remove the indigo dye. WO 94/29510, WO 96/18770, DE 196 12 194 A1 and DE 44 45 088 A1 describe enzymatic delignification processes which use enzymes together with mediators. In general, the disclosed mediators are compounds with the NO-, NOH- or HRNOH structure. These systems, of course, are restricted to use in pulp bleaching. Because the mechanisms underlying lignin-removing pulp bleaching, and this is the process involved here, are entirely different from those

underlying the decolorization, removal and/or "destruction" of denim dyes, particularly indigo dyes, in the jeans producing sector, it is entirely surprising that a number of substances of the said NO-, NOH- and HNROH types are also suitable for this application.

WO 97/06244 describes systems for the bleaching of pulp, for dye transfer inhibition and for bleaching stains when used with detergents, which systems employ enzymes (peroxidases, laccases) and enzyme-enhancing (hetero-)aromatic compounds, such as nitroso compounds etc.. In this case, as in patents WO 94/12619, WO 94/12620 and WO 94/12621, only the above-described use is intended. The mechanisms of stain decolorization in detergent bleaching or of dye transfer inhibition are entirely different from those underlying the decolorization, removal and/or "destruction" of indigo dyes, as, for example, in denim treatment. Hence, it is quite surprising that a number of substances of the said NO-, NOH- and HNROH-types are also suitable for this application.

Processes are known from said WO 94/12619, WO 94/12620 and WO 94/12621 in which the activity of peroxidase is increased by use of enhancers. Such enhancers are characterized in WO 94/12620 in terms of their half-life. According to WO 94/12621, enhancers are characterized by the formula  $A=N-N=B$  where N means nitrogen and A and B are defined cyclic groups. According to WO 94/12621, enhancers are organic chemicals containing at least two aromatic rings of which at least one is substituted with defined groups. All three applications concern (as already stated) dye transfer inhibition and the use of enhancers together with peroxidases as detergent additives or detergent compositions for washing applications or in cellulose bleaching. The combinations of these enhancers are restricted to use with peroxidases.

Moreover, oxidoreductases, primarily laccases, but also peroxidases, are currently used, mainly for treating denim for jeans.

It is known from patent application WO 96/12846 that laccase and peroxidase + certain enhancers, mainly derivatives of phenothiazine or phenoxazine, are used in two application forms in the treatment of cellulose-containing fabrics, such as cotton, viscose, rayon (artificial silk), ramie, linen, Tencel, silk or mixtures thereof or mixtures of these fabrics with synthetic fibers, for example a mixture of cotton and spandex (stretch denim), but mainly denim fabrics (mainly for use in jeans).

On the one hand, the system (oxidoreductases + enhancers) is intended as a replacement for the conventional hypochlorite bleaching of denim, usually after a stone washing pretreatment, this enzymatic treatment providing only partial replacement of hypochlorite, because the desired bleaching effect cannot be attained.

On the other hand, the system can be used together with cellulase in stone washing in place of the usual mechanical treatment with pumice, and this represents an improvement over the "treatment with cellulase only".

The main drawbacks of the system described in WO 96/12846 are the following, among others:

- 1) To achieve the desired goal, laccase must be used in considerable amounts (about 10 international units [IU]/g of denim);
- 2) In some cases, optimum treatment requires 2-3 hours;

- 3) The preferred mediator (here phenothiazine-10-propionic acid) must be used in an amount of about 2 to about 14 mg per gram of denim, which represents a considerable cost;
- 4) Buffer systems (about 0.1 mol/L) must be used, because otherwise no performance can be achieved, and this also raises the cost of the system. **This, for example, is not required for the system of the invention;**
- 5) The color of the enhancer component (long-lived radical) causes "browning" of the fabric.

The general advantage of a laccase and/or oxidoreductase system with enzyme action-enhancing compounds (enhancers, mediators etc.) when used in the above-described treatment of textiles (for example, jeans fabrics) consists, in an improved system over the prior-art systems, in that fashion looks can be achieved, which is not possible with conventional hypochlorite bleaching.

The dyes normally used for jeans denim are vat dyes, such as indigo or indigo derivatives, for example thioindigo, as well as sulfur dyes. By use of such special enzymatic systems, it is possible (as a result of the high specificity of such systems), when a mixed dye system such as an indigo dye and a sulfur dye system is present, to decolorize only the indigo dye, while the sulfur dye is not oxidized. Depending on the enzyme action-enhancing compound used, this can produce almost any desired fabric color (for example, gray shades etc), which is often desirable.

An additional advantage is that the enzymatic treatment is substantially more gentle than bleaching with hypochlorite, and as a result fiber damage is reduced. In the stone washing process, the ecological effect is of particular importance (in addition to the reduced fiber damage caused by enzymes) considering, for example, that this purely mechanical process produces about 1 kg of stone sludge per kg of jeans denim.

As can be seen from the prior art, for colored fabrics, in particular, the textile industry has a great need for alternative bleaching processes (alternatives to conventional hypochlorite bleaching) and/or treatment methods as alternatives to stone washing to achieve the bleached look, in the latter case also because of the environmental pollution problems.

The present invention has for an object to minimize or eliminate the drawbacks of the conventional processes: stone washing/bleaching after stone washing or general bleaching of dyed and/or undyed textile fabrics, particularly the pollution problems and fiber damage, as well as the drawbacks of the known oxidoreductase/enhancer systems (for example also NO-radical formation etc).

Entirely surprisingly, we have now found that the enzyme component System (ECS) of the invention exceeds the performance of the aforesaid oxidoreductase-mediator systems and that it does not exhibit the said drawbacks of the prior art.

In other words, according to the invention, the foregoing objective is reached by providing an enzyme component system (ECS) according which contains one or more lipases, preferably from the group of triacylglycerol lipases (3.1.1.3), or one or more amidases, preferably from the group of amidases (3.5.1.4) (**system component 1**) which from one or more fatty acids present (preferably C<sub>6</sub>-C<sub>26</sub> and particularly C<sub>8</sub>-C<sub>16</sub> fatty acids) (**system component 2**) and in the presence of an oxidant such as a peroxide, preferably

preferably from the group of amidases (3.5.1.4) (**system component 1**) which from one or more fatty acids present (preferably C<sub>6</sub>- C<sub>26</sub> and particularly C<sub>8</sub> – C<sub>16</sub> fatty acids) (**system component 2**) and in the presence of an oxidant such as a peroxide, preferably H<sub>2</sub>O<sub>2</sub> (**system component 3**) can produce peracids and in the presence of ketones as additional component (**system component 4**), for example, dioxiranes.

#### Description of Individual System Components of the Enzyme Component System (ECS) of the Invention

##### **System Component 1 (Lipases and Other Enzymes)**

Preferred are enzymes of group 3 (hydrolases), 3.1, 3.1.1, 3.1.2, 3.1.3, 3.1.4 and 3.1.7 according to the International Enzyme Nomenclature: Committee of the International Union of Biochemistry and Molecular Biology (Enzyme Nomenclature, Academic Press, Inc., 1992, pp. 306-337).

Preferred are enzymes acting on ester bonds (3.1, particularly those acting on carboxylate esters (3.1.1):

##### **A) Carboxylate Ester Hydrolases (3.1.1)**

- 3.1.1.1 Carboxylate esterase
- 3.1.1.2 Aryl esterase
- 3.1.1.3 Triacylglycerol lipase
- 3.1.1.4 Phospholipase A<sub>2</sub>
- 3.1.1.5 Lysophospholipase
- 3.1.1.6 Acetyl esterase
- 3.1.1.7 Acetylcholine esterase
- 3.1.1.8 Choline esterase
- 3.1.1.10 Tropine esterase
- 3.1.1.11 Pectin esterase
- 3.1.1.13 Sterol esterase
- 3.1.1.14 Chlorophyllase
- 3.1.1.15 L-Arabinolactonase
- 3.1.1.17 Gluconolactonase
- 3.1.1.19 Uronolactonase
- 3.1.1.20 Tannase
- 3.1.1.21 Retinyl palmitate esterase
- 3.1.1.22 Hydroxybutyrate dimer hydrolase
- 3.1.1.23 Acylglycerol lipase
- 3.1.1.24 3-Oxoadipate enol lactonase
- 3.1.1.25 1,4-Lactonase
- 3.1.1.26 Galactolipase
- 3.1.1.27 4-Pyrodioxolactonase
- 3.1.1.28 Acylcarnitine hydrolase
- 3.1.1.30 D-Arabinone lactonase
- 3.1.1.31 6-Phosphogluconolactonase
- 3.1.1.32 Phospholipase A<sub>1</sub>
- 3.1.1.32 6-Acetylglucose deacetylase
- 3.1.1.34 Lipoprotein lipase
- 3.1.1.35 Dihydrocoumarin hydrolase

3.1.1.36	Limonine D-ring lactonase
3.1.1.37	Steroid lactonase
3.1.1.38	Triacetate lactonase
3.1.1.39	Actinomycin lactonase
3.1.1.40	Orseilinate depside hydrolase
3.1.1.41	Cephalosporin C deacetylase
3.1.1.42	Chlorogenate hydrolase
3.1.1.43	$\alpha$ -Amino acid esterase
3.1.1.44	Methyloxaloacetate esterase
3.1.1.45	Carboxymethylenebutenolidase
3.1.1.46	Deoxylimonate A-ring lactonase
3.1.1.47	1-Alkyl-2-acetyl-glycerophosphocholine esterase
3.1.1.48	Fusarinine C ornithine esterase
3.1.1.49	Sinapine esterase
3.1.1.50	Wax ester hydrolase
3.1.1.51	Phorbol diester hydrolase
3.1.1.52	Phosphatidylinositol deacetylase
3.1.1.53	Sialate O-acetyl esterase
3.1.1.54	Acetoxybutyrylthiophene deacetylase
3.1.1.55	Acetylsalicylate deacetylase
3.1.1.56	Methylumbelliferyl acetate deacetylase
3.1.1.57	2-Pyrone-4,6-dicarboxy-gallate lactonase
3.1.1.58	N-Acetyl-galactosaminoglycan deacetylase
3.1.1.59	Juvenile hormone esterase
3.1.1.60	Bis(2-ethylhexyl)phthalate esterase
3.1.1.61	Protein glutamate methylesterase
3.1.1.63	11-cis-Retynil palmitate hydrolase
3.1.1.64	all-trans-Retynil palmitate hydrolase
3.1.1.65	L-Rhamnono-1,4-lactonase
3.1.1.66	5-(3,4-diacetoxybutyryl)-2,2'-bithiophene deacetylase
3.1.1.67	Fatty acid ethyl ester synthase
3.1.1.68	Xylono-1,4-lactonase
3.1.1.69	N-Acetylglucosaminylphosphatidylinositol deacetylase
3.1.1.70	Cetraxate benzyl esterase

Also preferred are:

#### **B) Thiol ester hydrolases (3.1.2)**

3.1.2.6	Hydroxyacylglutathione hydrolase
3.1.2.7	Glutathione thiol esterase
3.1.2.12	S-Formylglutathione hydrolase
3.1.2.13	S-Succinylglutathione hydrolase
3.1.2.14	Oleoyl-(acyl carrier protein) hydrolase
3.1.2.15	Ubiquitin thiol esterase
3.1.2.16	(Citrate-(pro-3S)-lyase)thiol esterase.

Also preferred are:

#### **C) Phosphoric Acid Monoester Hydrolases (Phosphatases) (3.1.3)**



- 3.1.3.1 Alkaline phosphatase
- 3.1.3.2 Acid phosphatase
- 3.1.3.3 Phosphoserine phosphatase
- 3.1.3.4 Phosphatidate phosphatase
- 3.1.3.8 3-Phytase
- 3.1.3.9 Glucose-6-phosphatase
- 3.1.3.10 Glucose-1-phosphatase
- 3.1.3.11 Fructose biphosphatase
- 3.1.3.12 Trehalose phosphatase
- 3.1.3.13 Bisphosphoglycerate phosphatase
- 3.1.3.14 Methylphosphothioglycerate phosphatase
- 3.1.3.15 Histidinol phosphatase
- 3.1.3.16 Phosphoprotein phosphatase
- 3.1.3.17 (Phosphorylase) phosphatase
- 3.1.3.18 Phosphoglycolate phosphatase
- 3.1.3.19 Glycerol-2-phosphatase
- 3.1.3.20 Phosphoglycerate phosphatase
- 3.1.3.21 Glycerol-1-phosphatase
- 3.1.3.22 Mannitol-1-phosphatase
- 3.1.3.23 Sugar phosphatase
- 3.1.3.24 Sucrose phosphatase
- 3.1.3.25 Myoinositol-1 (or 4)-monophosphatase
- 3.1.3.26 6-Phytase
- 3.1.3.27 Phosphatidylglycerophosphatase
- 3.1.3.36 Phosphatidylinositol biphosphatase
- 3.1.3.37 Sedoheptulose biphosphatase
- 3.1.3.38 3-Phosphoglycerate phosphatase
- 3.1.3.39 Streptomycin-6-phosphatase
- 3.1.3.40 Guanidinodeoxy-scylo-inositol-4-phosphatase
- 3.1.3.41 4-Nitrophenyl phosphatases
- 3.1.3.42 (Glycogen synthase-D) phosphatase
- 3.1.3.43 (Pyruvate dehydrogenase (lipoamide) phosphatase
- 3.1.3.44 3-Deoxy-manno-octulosonate-8-phosphatase
- 3.1.3.46 Fructose-2,6-bisphosphate-2-phosphatase
- 3.1.3.48 Protein-tyrosine phosphatase
- 3.1.3.49 (Pyruvate kinase) phosphatase
- 3.1.3.50 Sorbitol-6-phosphatase
- 3.1.3.51 Dolichyl phosphatase
- 3.1.3.52 3-Methyl-2-oxobutanoate dehydrogenase) (lipoamide) phosphatase
- 3.1.3.53 Myosin light chain phosphatase
- 3.1.3.54 Fructose-2,6-bisphosphate-6-phosphatase
- 3.1.3.55 Caldesmon phosphatase
- 3.1.3.56 Inositol-1,4,5-triphosphate-B-phosphatase
- 3.1.3.57 Inositol-1,4-bisphosphate-1-phosphatase
- 3.1.3.58 Sugar terminal phosphatase
- 3.1.3.59 Alkylacetyl glycerophosphatase
- 3.1.3.60 Phosphoenolpyruvate phosphatase
- 3.1.3.61 Inositol-1,4,5-trisphosphate-1-phosphatase
- 3.1.3.62 Inositol-1,3,4,5-tetrakisphosphate-3-phosphatase
- 3.1.3.63 2-Carboxy-D-arabinitol-1-phosphatase

Also preferred are:

#### **D) Phosphoric Acid Diester Hydrolases (3.1.4)**

- 3.1.4.1 Phosphodiesterase I
- 3.1.4.2 Glycerophosphocholine phosphodiesterase
- 3.1.4.3 Phospholipase C
- 3.1.4.4 Phospholipase D
- 3.1.4.10 1-Phosphatidylinositol phosphodiesterase
- 3.1.4.11 1-Phosphatidylinositol-4,5-bisphosphate phosphodiesterase
- 3.1.4.12 Sphingomyelin phosphodiesterase
- 3.1.4.13 Serine-ethanolamine phosphate phosphodiesterase
- 3.1.4.14 (Acyl carrier protein) phosphodiesterase
- 3.1.4.36 1,2-Cyclic inositol phosphate phosphodiesterase
- 3.1.4.38 Glycerophosphocholine choline phosphodiesterase
- 3.1.4.39 Alkylglycerophosphoethanolamine phosphodiesterase
- 3.1.4.40 CMP-N-acylneuraminate phosphodiesterase
- 3.1.4.41 Sphingomyelin phosphodiesterase D
- 3.1.4.42 Glycerol-1,2-cyclic phosphate-2-phosphodiesterase
- 3.1.4.43 Glycerophosphoinositol inositol phosphodiesterase
- 3.1.4.44 Glycerophosphoinositol glycerophosphodiesterase
- 3.1.4.45 N-acetylglucosamine-1-phosphodiesterase
- 3.1.4.46 Glycerophosphodiester phosphodiesterase
- 3.1.4.47 Variant surface glycoprotein phospholipase C
- 3.1.4.48 Dolichyl phosphate-glucose phosphodiesterase
- 3.1.4.49 Dolichyl phosphate-mannose phosphodiesterase
- 3.1.4.50 Glycoprotein phospholipase D
- 3.1.4.51 Glucose-1-phospho-D-mannosylglycoprotein phosphodiesterase.

Also preferred are:

#### **E) Diphosphoric Acid Monoster Hydrolases (3.1.7)**

- 3.1.7.1 Prenyl pyrophosphatase
- 3.1.7.3 Monoterpenyl pyrophosphatase

Particularly preferred among these are enzymes of group 3.1.1.3 lipases (triacylglycerol lipases, triglycerolacyl hydrolases) from organisms such as *Candida antarctica*, *Candida rugosa*, *Candida lipolytica*, *Candida cylindracea*, *Candida spec.*, *Geotrichum candidum*, *Humicola lanuginosa*, *Penicillium cambertii*, *Penicillium roquefortii*, *Aspergillus spec.*, *Mucor javanicus*, *Mucor mehei*, *Rhizopus arrhizus*, *Rhizopus niveus*, *Rhizopus delamar*, *Rhizopus spec.*, *Chromobacterium viscosum*, *Pseudomonas cepacia* and *Pseudomonas spec.* from wheat seedlings or pancreas (pig or other sources) or other sources.

**Enzymes splitting carbon/nitrogen (C/N) bonds (other than peptide bonds) can also be used (3.5).**

This subclass includes enzymes capable of splitting amides, amidines and other C/N bonds. Particularly preferred are enzymes of class 3.5.1 which act on linear amides, of class 3.5.2 which act on cyclic amides, of class 3.5.3 which act on linear amidines, of class 5.3.1 which act on nitriles and of class 3.5.99 which act on other compounds.

Particularly preferred are enzymes of class 3.5.1. which act on linear amides:

- 3.5.1.1 Asparaginase
- 3.5.1.2 Glutaminase
- 3.5.1.3  $\omega$ -Amidase
- 3.5.1.4 Amidase
- 3.5.1.5 Urease
- 3.5.1.6  $\beta$ -Ureidopropionase
- 3.5.1.7 Ureidosuccinase
- 3.5.1.8 Formylaspartate deformylase
- 3.5.1.9 Arylformamidase
- 3.5.1.10 Formyltetrahydrofolate deformylase
- 3.5.1.11 Penicillin amidase
- 3.5.1.12 Biotinidase
- 3.5.1.13 Arylacyl amidase
- 3.5.1.14 Aminoacylase
- 3.5.1.15 Aspartoacylase
- 3.5.1.16 Acetylornithine deacetylase
- 3.5.1.17 Acyl-lysine deacetylase
- 3.5.1.18 Nicotinamidase
- 3.5.1.20 Citrullinase
- 3.5.1.22 Pantothenase
- 3.5.1.30 5-Aminopentanamidase
- 3.5.1.31 Formylmethionine deformylase
- 3.5.1.32 Hippurate hydrolase
- 3.5.1.39 Alkylamidase
- 3.5.1.40 Acylagmatin amidase
- 3.5.1.41 Chitin deacetylase
- 3.5.1.42 Nicotinamide nucleotide amidase
- 3.5.1.49 Formamidase
- 3.5.1.50 Pentanamidase
- 3.5.1.55 Long-chain fatty acylglutamate deacylase
- 3.5.1.56 N,N-Dimethylformamidase
- 3.5.1.57 Tryptophanamidase
- 3.5.1.58 N-Benzyloxycarbonylglycine hydrolase

3.5.1.75 Urethanase

Also preferred are enzymes of class 3.5.2 which act on cyclic amides, such as:

- 3.5.2.1 Barbiturase
- 3.5.2.2 Dihydropyrimidase
- 3.5.2.3 Dihydroorotase
- 3.5.2.4 Carboxymethylhydantoinase
- 3.5.2.5 Allantoinase
- 3.5.2.6  $\beta$ -Lactamase
- 3.5.2.10 Creatininase

Also particularly preferred are class 3.5.3 enzymes which act on linear amidines, such as:

- 3.5.3.1 Arginase
- 3.5.3.3 Creatinase
- 3.5.3.4 Allantoinase
- 3.5.3.6 Arginine deiminase
- 3.5.3.9 Allantoate deiminase
- 3.5.3.10 D-Arginase
- 3.5.3.14 Amidinoaspartase
- 3.5.3.15 Protein-arginine deiminase

Also particularly preferred are enzymes of class 3.5.4 which act on cyclic amidines, such as:

- 3.5.4.8 Aminoimidazolase
- 3.5.4.21 Creatinine deaminase

Preferred are also the enzymes of class 3.5.99 which act on other compounds, such as:

- 3.5.99.1 Riboflavinase
- 3.5.99.2 Thaminase

Particularly preferred enzymes are especially those of class 3.5.5. 1, nitrilase (3.5.5.2 - 3.5.5.6, other nitrilases).

Also particularly preferred are enzymes of class 3.5.1, here particularly those of class 3.5.1.4, amidases.

**System Component 2 of the Enzyme Component System (ECS) of the Invention**

Fatty acids which in the process according to the invention can be used as sources of peracids are, for example:

### **System Component 2 of the Enzyme Component System (ECS) of the Invention**

Fatty acids which in the process according to the invention can be used as sources of peracids are, for example:

#### **1) Saturated fatty acids**

Butanoic acid	(butyric acid)
Pentanoic acid	(valeric acid)
Hexanoic acid	(caproic acid)
Heptanoic acid	(enanthic acid)
Octanoic acid	(caprylic acid)
Nonanoic acid	(pelargonic acid)
Decanoic acid	(capric acid)
Undecanoic acid	
Dodecanoic acid	(lauric acid)
Tridecanoic acid	
Tetradecanoic acid	(myristic acid)
Pentadecanoic acid	
Hexadecanoic acid	(palmitic acid)
Heptadecanoic acid	
Octadecanoic acid	(stearic acid)
Nonadecanoic acid	
Eicosanoic acid	(arachic acid)
Heneicosanoic acid	
Docosanoic acid	(behenic acid)
Tricosanoic acid	
Tetracosanoic acid	(lignoceric acid)
Pentacosanoic acid	
Hexacosanoic acid	(cerotic acid)
Octacosanoic acid	
Triacosanoic acid	(melissic acid)

#### **2) Unsaturated fatty acids**

10-Undecenoic acid	
9-cis-Dodecenoic acid	(lauroleic acid)
9-cis-Tetradecenoic acid	(myristoleic acid)
9-cis-Hexadecenoic acid	(paimitoleic acid)
6-cis-Octadecenoic acid	(petroselic acid)
6-trans-Octadecenoic acid	(petroselaidic acid)
9-cis-Octadecenoic acid	(oleic acid)
9-trans-Octadecenoic acid	(elaidic acid)
9-cis, 12 cis-Octadecadienoic acid	(linoleic acid)
9-trans, 1 2-trans-Octadecadienoic acid	(linolaidic acid)
9-cis, 12-cis, 15-cis-Octadecatrienoic acid	(linolenic acid)
9-trans, 11 -trans, 1 3-trans- Octadecatrienoic acid	( $\alpha$ -eleostearic acid)
9-cis, 11 -trans, 1 3-trans-Octadecatrienoic acid	( $\beta$ -eleostearic acid)

4,8,12,15,19-Docosapentaenoic acid

(clupanodonic acid)

### 3) Polyunsaturated fatty acids

9,12-Octadecadienoic acid

(linoleic acid)

9,12,15-Octadecatrienoic acid

(linolenic acid)

5,9,12-Octadecatrienoic acid

(eleostearic acid)

9,11,13-Octadecatrienoic acid

(parinaric acid)

9,11,13,15-Octadecatetraenoic acid

5,11,14-Icosatrienoic acid

(arachidic acid)

5,8,11,14-Icosatetraenoic acid

4,8,12,15,18-Icosapentaenoic acid

(clupanodonic acid)

4,8,12,15,19-Docosapentaenoic acid

(nisinic acid)

4,8,12,15,18,21-Tetracosahexaenoic acid

Particularly preferred are tetradecanoic acid (myristic acid) and dodecanoic acid (lauric acid).

### **System Component 3 (Oxidants: Peroxides or Per Compounds) of the Enzyme Component System (ECS) of the Invention**

Preferred oxidants in the enzyme component system of the invention are hydrogen peroxide ( $H_2O_2$ ), organic peroxides and per-compounds such as perborates, persulfates, percarbonates, perphosphates, percarbamides, perchlorates etc.

Preferred organic peroxides are, for example:

3-chloroperoxybenzoic acid, monoperoxyphthalic acid Mg salt, di-tert.butyl peroxide, cumene hydroperoxide, lauroyl peroxide, chloroperoxybenzoic acid, dicumyl peroxide, methyl ethyl ketone peroxide, benzoyl peroxide, diperoxidododecandionic acid Na salt etc..

Besides lipase-catalyzed peracid formation, combinations of bleach activators, such as TAED (tetraacetylenediamine), TAGU (tetraacetylglycoluril) and iso-NOBS (sodium p-isononanoyl-oxybenzenesulfonate) and the like, which are also used in detergents, together with per-compounds such as perborates, percarbonates etc. can serve as additional sources of peracid generation.

The abovesaid per-compounds, as well as, for example, glucose + GOD, can be used as systems generating  $H_2O_2$  for the corresponding lipase action. Substances such as nitrilamines or dicyandiamines or metal ions, e.g.  $Mo^{6+}$ ,  $Va^{6+}$  and  $W^{6+}$  can be used together with a peroxide, for example  $H_2O_2$ .

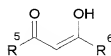
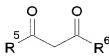
### **System Component 4 (Ketones) of the Enzyme Component System (ECS) of the Invention**

Particularly preferred are carbonyl compounds of general formula I:



The R<sup>1</sup> and R<sup>2</sup> groups can be equal or different and denote aliphatic or aromatic groups. Moreover, the R<sup>1</sup> and R<sup>2</sup> groups can form a ring containing besides carbon also heteroatoms such as nitrogen, oxygen and sulfur.

Particularly preferred are 1,2-diketones (formula II), 1,3-diketones (formula III), polyketones (polyketides) and the tautomeric enols (formula IV):



wherein the R<sup>3</sup> to R<sup>6</sup> groups, once again, can be equal or different and denote aliphatic or aromatic groups. Moreover, groups R<sup>3</sup> and R<sup>4</sup> and groups R<sup>5</sup> and R<sup>6</sup>, together, can form a ring containing besides carbon also heteroatoms such as nitrogen, oxygen or sulfur. The possibility of tautomerization or formation of a resonance hybrid is particularly important in this case.

Besides general carbonyl compounds, particularly preferred are ketones, such as, in general hydroxyketones,  $\alpha,\beta$ -unsaturated ketones, oxycarboxylic acids, quinones and halogenated ketones.

Particularly preferred among these are the following:

Acetone, methyl ethyl ketone, diethyl ketone, methyl n-butyl ketone, methyl isobutyl ketone, cyclohexanone, cyclopentanone, 2-methylcyclohexanone, 3-methylcyclohexanone, 4-methylcyclohexanone, dihydroxyacetone, diacetyl monohydrazone, diacetyl dihydrazone, acetophenone, p-hydroxyacetophenone, 1-phenyl-3-butanone, 3-pentanone, 4-heptanone, 2-nonanone, cycloheptanone, cyclodecanone, cyclodecanone, cyclododecanone, dimethyl ketone, ethyl propyl ketone, methyl amyl ketone, acetylacetone, pinacolone, methyl isopropyl ketone, methyl isoamyl ketone, ethyl amyl ketone diisopropyl ketone, diisobutyl ketone, methyl vinyl ketone, methyl isopropenyl ketone, mesityl oxide, isophorone, hydroxyacetone, methoxyacetone, 2,3-pentanedione, 2,3-hexanedione, phenylacetone, propiophenone, benzophenone, benzoin, benzil, 4,4'-dimethoxybenzil, 4'-methoxyacetophenone, 3'-methoxyacetophenone, O-ethylbenzoin, (2-methoxyphenyl)acetone, (4-methoxyphenyl)acetone, methoxy-2-propanone, glyoxylic acid, benzyl glyoxylate, benzylacetone, methyl benzyl ketone, methylcyclohexyl ketone, 2-decanone, dicyclohexyl ketone, 3,3-dimethyl-2-butanone, methyl isobutyl ketone, methyl isopropyl ketone, 2-methyl-3-heptanone, 5-methyl-3-heptanone, 6-methyl-5-hepten-2-one, 5-methyl-2-hexanone, 3-nonanone, 5-nonanone, 2-octanone, 3-octanone, 2-undecanone, 1,3-dichloroacetone, 1-hydroxy-2-butanone, 3-hydroxy-2-butanone, 4-hydroxy-4-methyl-2-pentanone, 2-(1S)-adamantanone, anthrone, bicyclo(3.2.0)hept-2-en-6-one, cis-bicyclo(3.3.0)octan-3,7-dione, (1S)-(-)-camphor, p-chloranil, cyclobutanone, 1,3-cyclohexanedione, 1,4-cyclohexanedione monoethylene ketal, dibenzosuberone, ethyl 4-oxocyclohexanecarboxylate, 9-fluorenone, 1,3-indandione, methylcyclohexanone, phenylcyclohexanone, 4-propylcyclohexanone, 1,2,3,4-tetrahydro-1-naphthalenone, 1,2,3,4-tetrahydro-2-naphthalenone, 3,3,5-trimethylcyclohexanone, 3-acetoxy-2-cyclohexen-1-one, benzylideneacetone, (R)-(-)-carvone, (S)-(+)-carvone, curcumin, 2-cyclohexen-1-one, 2,3-diphenyl-2-cyclopropen-1-one, 2-hydroxy-3-methyl-2-cyclopenten-1-one, isophorone,  $\alpha$ -ionone,  $\beta$ -ionone, 3-methoxy-2-cyclohexen-1-one, 3-methyl-2-cyclopenten-1-one, 3-methyl-3-penten-2-one, (R)-(+)-pulegone, tetraphenyl-2,4-cyclopentadien-1-one, 2,6,6-trimethyl-2-cyclohexen-1,4-dione, 2-acetylbenzoic acid, 1-acetylnaphthalene, 2-acetylnaphthalene, 3'-aminoacetophenone, 4'-aminoacetophenone, 4'-cyclohexylacetophenone, 3',4'-diacetoxyacetophenone, diacetylbenzene, 2',4'-dihydroxyacetophenone, 2',5'-dihydroxyacetophenone, 2',6'-dihydroxyacetophenone, 3,4-dimethoxyacetophenone, 2'-hydroxyacetophenone, 4'-hydroxyacetophenone, 3'-methoxyacetophenone, 4'-methoxyacetophenone, 2'-methylacetophenone, 4'-methylacetophenone, 2'-nitroacetophenone, 3'-nitroacetophenone, 4'-phenylacetophenone, 3',4',5'-trimethoxyacetophenone, 4'-aminopropiophenone, benzoylacetone, benzoylpropionic acid, benzylideneacetophenone, cyclohexyl phenyl ketone, desoxybenzoin, 4',4'-dimethoxybenzil, 1,3-diphenyl-1,3-propanedione, ethylbenzoyl acetate, ethyl phenylglyoxylate, 4'-hydroxypropiophenone, 1,3-indandione, 1-indanone, isopropyl phenyl ketone, 6-methoxy-1,2,3,4-tetrahydronaphthalen-1-one, methylphenyl glyoxylate, phenylglyoxylonitrile, 1-phenyl-1,2-propanedione 2-oxime, valerophenone, 2-acetyl- $\gamma$ -butyrolactone, 2-acetylpyrrole, 1-benzylpiperidin-4-one, dehydroacetic acid, 3,4-dihydro-4,4-dimethyl-2H-pyran-2-one, 1,4-dihydro-4-pyridinone, N-ethoxycarbonyl-4-piperidinone, 2-methyl furyl ketone, 5-hydroxy-2-hydroxymethyl-4H-



pyran-4-one, 3-hydroxy-2-methyl-4-pyranone, 3-indolyl methyl ketone, isatin, 1-methyl-4-piperidinone, methyl 2-pyridyl ketone, methyl 3-pyridyl ketone, methyl 4-pyridyl ketone, methyl 2-thienyl ketone, phenyl 2-pyridyl ketone, phenyl 4-pyridyl ketone, tetrahydrofuran-2,4-dione, tetrahydro-4H-pyran-4-one, 2,2,6,6-tetramethyl-4-piperidone, xanthone, acenaphthene quinone, pyruvic acid, (1 R)-(-)-camphor quinone, (1S)-(+)-camphor quinone, 3,5-ditert-butyl-o-benzoquinone, 1,2-dihydroxy-3,4-cyclobutenedione, ethyl (2-amino-4-thiazolyl)glyoxylate, ethyl pyruvate, 2,3-hexanedione, 3,4-hexanedione, 3-methyl-2-oxobutyric acid, 3-methyl-2-oxovaleric acid, 4-methyl-2-oxovaleric acid, 2-oxobutyric acid, 2,3-pentanedione, 9,10-phenanthrene quinone, acetoacetanilide, 2-acetyl- $\gamma$ -butyrolactone, 2-acetylcyclopentanone, allyl acetoacetate, benzoylacetone, tert-butyl acetoacetate, 1,3-cyclopentanedione, diethyl 3-oxoglutarate, dimethyl acetylsuccinate, dimethyl 3-oxoglutarate, 1,3-diphenyl-1,3-propanedione, ethyl acetoacetate, ethyl benzoylacetate, ethyl butyrylacetate, ethyl 2-oxocyclohexanecarboxylate, ethyl 2-phenylacetoacetate, methyl acetoacetate, 2-methyl-1,3-cyclohexanedione, 2-methyl-1,3-cyclopentanedione, methyl isobutyrylacetate, methyl 3-oxopentanoate, methyl pivaloylacetate, 3-oxoglutaric acid, tetrahydrofuran-2,4-dione, 2,2,6,6-tetramethyl-3,5-heptanedione, 3-benzoylpropionic acid, 1,4-cyclohexanedione, dimethyl acetylsuccinate, ethyl levulinate, 2-aminoanthraquinone, anthraquinone, p-benzoquinone, 1,4-dihydroxyanthraquinone, 1,8-dihydroxyanthraquinone, 2-ethylantraquinone, methyl-p-benzoquinone, 1,4-naphthoquinone, tetramethyl-p-benzoquinone, 2,2-dimethyl-1,3-dioxan-4,6-dione, 2-benzoylbenzoic acid, 3-benzoylpropionic acid, 5,6-dimethoxyphthalaldehydic acid, levulinic acid, methyl trans-4-oxo-2-pentenoate, phthalaldehydic acid, terephthalaldehydic acid, dibutyl maleate, dibutyl succinate, dibutyl phthalate, dicyclohexyl phthalate, diethyl acetamidomalonate, diethyl adipate, diethyl benzylmalonate, diethyl butylmalonate, diethylethoxymethylenemalonate, diethyl ethylmalonate, diethyl fumarate, diethyl glutarate, diethyl isopropylidenemalonate, diethyl maleate, diethyl malonate, diethyl methylmalonate, diethyl oxalate, diethyl 3-oxoglutarate, diethyl phenylmalonate, diethyl phthalate, diethyl pimelate, diethyl sebacate, diethyl suberate, diethyl succinate, diisobutyl phthalate, dimethyl acetylene-dicarboxylate, dimethyl acetylsuccinate, dimethyl adipate, dimethyl 2-aminoterephthalate, dimethyl fumarate, dimethyl glutaconate, dimethyl glutarate, dimethyl isophthalate, dimethyl malonate, dimethylmethoxymalonate, dimethyl methylenesuccinate, dimethyl oxalate, dimethyl 3-oxoglutarate, dimethyl phthalate, dimethyl succinate, dimethyl terephthalate, ethylene glycol diacetate, ethylene glycol dimethacrylate, monoethyl fumarate, monomethyl malonate, monoethyl adipate, monomethyl phthalate, monomethyl pimelate, monomethyl terephthalate, 1,2-propylene glycol diacetate, triethyl methanetricarboxylate, trimethyl 1,2,3-propanetricarboxylate, 3-acetoxy-2-cyclohexen-1-one, allyl acetoacetate, allyl cyanoacetate, benzyl acetoacetate, tert-butyl acetoacetate, butyl cyanoacetate, chlorogenic acid hemihydrate, coumarin-3-carboxylic acid, diethyl ethoxycarbonylmethanephosphonate, dodecyl gallate, dodecyl 3,4,5-trihydroxybenzoate, (2,3-epoxypropyl) methacrylate, (2-ethoxyethyl) acetate, ethyl acetamidocycanoacetate, ethyl 2-aminobenzoate, ethyl 3-aminopyrazol-4-carboxylate, ethyl benzoylacetate, ethyl butyrylacetate, ethyl cyanoacetate, ethyl 2-cyano-3-ethoxyacrylate, ethyl cyanofornate, ethyl 2-cyanopropionate, ethyl 3,3-diethoxypropionate, ethyl 1,3-dithian-2-carboxylate, ethyl 2-ethoxyacetate, ethyl 2-furancarboxylate, ethyl levulinate, ethyl mandelate, ethyl gallate, ethyl 2-methyl lactate, ethyl 4-nitrocinnamate, ethyl oxamate, ethyl 2-

oxocyclohexanecarboxylate, ethyl 4-oxocyclohexane- carboxylate, ethyl 5-oxohexanoate, ethyl 2-phenylacetoacetate, ethyl 4-piperidinecarboxylate, ethyl 2-pyridinecarboxylate, ethyl 3-pyridinecarboxylate, ethyl 4-pyridinecarboxylate, ethyl thioglycolate, ethyl 3,4,5-trihydroxybenzoate, 2-hydroxyethyl methacrylate, 2-hydroxypropyl methacrylate, 3-indole acetate, 2-methoxyethyl acetate, 1-methoxy-2-propyl acetate, methyl 2- aminobenzoate, methyl 3-aminocrotonate, methyl cyanoacetate, methyl 4-cyanobenzoate, methyl 4-formylbenzoate, methyl 2-furancarboxylate, methyl isobutyrylacetate, methyl methoxyacetate, methyl 2-methoxybenzoate, methyl 3-oxopentanoate, methyl phenylglyoxylate, methyl phenyl- sulfinylacetate, methyl pivaloylacetate, methyl 3-pyridinecarboxylate, 5-nitrofurfurylidene diacetate, propyl gallate, propyl 3,4,5-trihydroxybenzoate, methyl 3-methylthiopropionate, acetamide, acetanilide, benzamide, benzanilide, N,N-diethylacetamide, N,N-dimethylformamide, N,N-diethyl-3-methyl- benzamide, diethyltoluamide, N,N-dimethylacetamide, N,N-diphenylacetamide, N-methylformamide, N-methylformanilide, N-acetylthiourea, adipic acid diamide, 2-aminobenzamide, 4-aminobenzamide, succinic acid diamide, malonic acid diamide, N,N'-methylene diacrylamide, oxalic acid diamide, pyrazine-2-carboxamide, pyridine-4-carboxamide, N,N,N',N'-tetramethylsuccinic acid diamide, N,N,N',N'-tetramethylglutaric acid diamide, acetoacetanilide, benzohydroxamic acid, cyanoacetamide, 2-ethoxybenzamide, diethyl acetamidomalonate, ethyl acetamidocycanoacetate, ethyl oxamate, hippuric acid Na salt, N-(hydroxymethyl)acrylamide, L-(-)-lactamide, 2'-nitroacetanilide, 3'- nitroacetanilide, 4'-nitroacetanilide, paracetamol, piperine, salicylanilide, 2-acetyl- $\gamma$ -butyrolactone,  $\gamma$ -butyrolactone,  $\epsilon$ -caprolactone, dihydrocoumarin, 4-hydroxycoumarin, 2-(5H)-furanone, 2,5-dihydro-5-methoxy-2-furanone, phthalide, tetrahydrofuran-2,4-dione, 2,2,6-trimethyl-1,3-dioxin-4-one,  $\gamma$ - valerolactone, 4-amino-1,3-dimethyluracil, barbituric acid, O-benzoyloxycarbonyl-N-hydroxysuccinimide, succinimide, 3,6-dimethyl-piperazin-2,5-dione, 5,5-diphenylhydantoin, ethyl 1,3-dioxoisindoline-2-carboxylate, 9-fluorenylmethylsuccinimidyl carbonate, hydantoin, maleimide, 3-methyl-1- phenyl-2-pyrazolin-5-one, 1-methyl-2-pyrrolidone, methyluracil, 6-methyluracil, oxindole, phenytoin, 1-(2H)-phthalazinone, phthalimide, 2,5-piperazinedione, 2-piperidinone, 2-pyrrolidone, rhodanine, saccharin, 1,2,3,6-tetrahydropthalimide, 1,2,3,4-tetrahydro-6,7-dimethoxyquinazolin-2,4-dione, 1,5,5-trimethyl-hydantoin, 1-vinyl-2-pyrrolidone, di-tert-butyl dicarbonate, diethyl carbonate, dimethyl carbonate, dimethyl dicarbonate, diphenyl carbonate, 4,5-diphenyl-1,3-dioxol-2-one, 4,6- diphenylthieno-(3,4-d)-1,3-dioxol-2-one 5,5-dioxide, ethylene carbonate, magnesium methoxide methyl carbonate, monomethyl carbonate Na salt, propenyl carbonate, N-allylurea, azodicarbonamide, N-benzylurea, biuret, 1,1'-carbonyldiimidazol, N,N-dimethylurea, N-ethylurea, N-formylurea, urea, N-methylurea, N-phenylurea, 4-phenylsemicarbazide, tetramethylurea, semicarbazide hydrochloride, diethyl azodicarboxylate, methyl carbamate, 1-(4-methoxyphenyl)-2-(2-methoxyphenoxy)ethanone and 1-(4-methoxyphenyl)-2-(2-methoxyphenoxy)ethanol.

Also preferred are anhydrides, such as the following:

Benzoic anhydride, benzene-1,2,4,5-tetracarboxylic acid-1,2,4,5-dianhydride, 3,3',4,4'-benzophenonetetracarboxylic anhydride, succinic anhydride, butyric anhydride, crotonic anhydride, cis-1,2-cyclo- hexanedicarboxylic anhydride, di-tert.butyl dicarbonate,

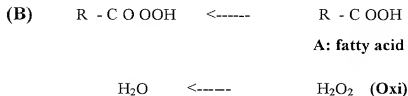
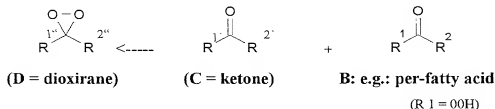
dimethyl dicarbonate, dodecenylsuccinic anhydride, Epicon B 4400, acetic anhydride, glutaric anhydride, hexanoic anhydride, isatoic anhydride, isobutyric anhydride, isovaleric anhydride, maleic anhydride, 1,8-naphthalenedicarboxylic anhydride, 3-nitrophthalic anhydride, 5-norbornene-2,3-dicarboxylic anhydride, phthalic anhydride, 2-phenylbutyric anhydride, pivalic anhydride, propionic anhydride, cis-1,2,3,6-tetrahydrophthalic anhydride and valeric anhydride.

Particularly preferred are benzophenones such as the following:

Benzophenone, 4-aminobenzophenone, 2-amino-5-chlorobenzophenone, benzophenone-2-carboxylic acid, (S)-(-)-2-(N-benzopropyl)aminobenzophenone, 4,4'-bis(dimethylamino)benzophenone, 4,4'-bis(diethylamino)benzophenone, 3,4-dimethoxybenzophenone, 4,4'-dihydroxybenzophenone, 2,4-dihydroxybenzophenone, 4-hydroxybenzophenone, 2-hydroxy-4-methoxybenzophenone, 4-methoxybenzophenone, 4,4'-dimethoxybenzophenone, 2,2',4,4'-tetrahydroxybenzophenone and 2-chlorobenzophenone.

Figure 1 shows schematically a possible reaction cycle involving all components.

Figure 1:



lipase/amidase

component 1) = enzyme/ hydrolase: e.g.: lipase/amidase

component 2) = fatty acid (A)

component 3) = oxidation agent, e.g.: H<sub>2</sub>O<sub>2</sub> (Oxi)

**B) = e.g.: per-fatty acid**

**D) = dioxirane**

A more detailed description of the enzyme component system (ECS) of the invention in terms of various applications follows:

### 1) Use in Wood Pulp Bleaching

One of the components of the enzyme component system (ECS) of the invention used is an enzyme, preferably lipase from, for example, *Humicola lanuginosa*, used at a concentration of 0.05 to 5 mg per gram of wood pulp, preferably from 0.05 to 2 mg of enzyme per gram of wood pulp (which corresponds to about 250 to 10,000 IU per gram of wood pulp) (1 IU hydrolyzes 1  $\mu$  equivalent of a triglyceride fatty acid in 1 hour at pH 7.7 and 37 °C).

Preferably, the delignification (bleaching) with the enzyme component system of the invention is carried out in the presence of oxygen or air at atmospheric pressure or at a slight positive O<sub>2</sub> pressure and at pH from 2 to 11, preferably at pH 3-9, at a temperature of 20 to 95 °C, preferably 40-95 °C and a pulp consistency of 0.5 to 40%. An unusual and surprising finding concerning the use of enzymes for wood pulp bleaching is that when the enzyme component system of the invention is used, the consistency of the material can be increased and the kappa value thus markedly reduced. For economic reasons, the process according to the invention is carried out at a pulp consistency from 4 to 35% and particularly from 4 to 15%.

Another component is the oxidant, preferably H<sub>2</sub>O<sub>2</sub>, which is used at a concentration from 0.05 to 20 mg/g of wood pulp (100% basis) and preferably from 0.05 to 10 mg/g of wood pulp.

Another component consists of one or more fatty acids, preferably C<sub>6</sub> to C<sub>26</sub>, and particularly C<sub>6</sub> to C<sub>16</sub> fatty acids and more particularly tetradecanoic or dodecanoic acid at a concentration from 0.05 to 20 mg/g of wood pulp and preferably at a concentration from 0.05 to 10 mg/g of wood pulp.

Another component are the ketones, preferably, for example, benzophenone at a concentration from 0.05 to 20 mg/g of wood pulp and preferably at a concentration from 0.05 to 10 mg/g of wood pulp.

When the enzyme component system of the invention is used, for example, in a process for treating lignin, the chosen components are mixed with an aqueous suspension of the lignin-containing pulp simultaneously or in any order. The reaction is preferably started by adding the oxidant or the enzyme.

Besides the abovesaid main components of the enzyme component system (ECS) of the invention, namely the enzymes (lipases/amidases), the oxidant, the fatty acids and the ketones, the bleaching system can also contain phenolic and/or nonphenolic compounds with one or more benzene rings which are capable of improving "oxidation transfer"

(redox cascade) and/or of scavenging the radicals which possibly could cause polymerization of the lignin.

Besides the abovesaid preferred oxidant,  $H_2O_2$ , particularly preferred are air, oxygen (possibly in addition to  $H_2O_2$ ), organic peroxides, percompounds such as sodium perborate and/or sodium percarbonate, persulfates etc. (optionally together with activators such as TAED, nitrilamines, dicyandiamines etc.). Oxygen can also be generated in situ by  $H_2O_2$  + catalase or the like, or  $H_2O_2$ , can be generated in situ from GOD + glucose or similar systems.

The efficacy of the enzyme component system (ECS) as the oxidation system of the invention in the modification, degradation or bleaching of lignin, lignin-containing materials or similar substances is often even enhanced when  $Mg^{2+}$  ions are present besides the said constituents. The  $Mg^{2+}$  ions can be derived, for example, from a salt such as  $MgSO_4$ . The  $Mg^{2+}$  concentration is in the range from 0.1 to 2 mg/g, and preferably from 0.2 to 0.6 mg/g, of lignin-containing material.

In some cases, the efficacy of the enzyme component system (ECS) of the invention can be increased even further by adding to the system besides  $Mg^{2+}$  ions also complexing agents, for example ethylenediaminetetraacetic acid (EDTA), diethylenetriaminopentaacetic acid (DTPA), hydroxyethylenediaminetriacetic acid (HEDTA), diethylenetriaminepentamethylenephosphonic acid (DTMPA), nitrilotriacetic acid (NTA), polyphosphoric acid (PPA) etc. The concentration of said complexing agents is in the range from 0.2 to 5 mg/g, and preferably from 1 to 3 mg/g, of the lignin-containing material.

Surprisingly, we have also found that for many wood pulps an acid wash (pH 2 to 6 and preferably 2 to 5) or a Q step (pH 2 to 6 and preferably 2 to 5) preceding the ECS step causes a marked decrease in kappa value compared to processing without this special pretreatment. In the Q step, chelators usually employed for this purpose (for example, EDTA or DTPA) are preferably used at a concentration from 0.1 to 1 % and particularly from 0.1 to 0.5 %.

Reducing agents can be added at the same time, which with the oxidants present give rise to a certain redox potential. Suitable reducing agents are sodium bisulfite, sodium dithionite, ascorbic acid, thio compounds, mercapto compounds or glutathione etc..

It is also possible to add to the system radical generators or radical scavengers (scavenging, for example, OH- or OOH- radicals). These can improve the interaction between the redox and the radical mediators.

Additional metal salt can also be added to the reaction solution. These are important in that they interact with chelators as radical generators or redox centers. In the reaction solution, the salts form cations. Such ions are, among others,  $Fe^{2+}$ ,  $Fe^{3+}$ ,  $Mn^{2+}$ ,  $Mn^{3+}$ ,  $Mn^{4+}$ ,  $Cu^{1+}$ ,  $Cu^{2+}$ ,  $Ca^{2+}$ ,  $Ti^{3+}$ ,  $Ce^{4+}$  and  $Al^{3+}$ .

The chelates present in the solution can also serve as mimicking substances for certain oxido- reductases, such as the laccases (copper complexes) or for the lignin or manganese peroxidases (heme complexes). By mimicking substances are meant substances simulating the prosthetic groups of (in the present case) oxidoreductases and, for example, capable of catalyzing oxidation reactions.

Moreover, NaOCl can be added to the reaction mixture. This compound can form singlet oxygen by interacting with hydrogen peroxide.

Finally, it is also possible to use detergents. These include nonionic, anionic, cationic and amphoteric surfactants. Detergents improve the penetration of the enzymes and other components into the fibers.

It may also be necessary to add polysaccharides and/or proteins to the reaction mixture. Suitable polysaccharides are, in particular, glucans, mannans, dextrans, levans, pectins, alginates or vegetable gums, and suitable proteins are gelatins and albumins. These substances serve mainly as protective colloids for the enzymes.

Other proteins that can be added are proteases such as pepsin, bromelain, papain etc. These substances can, among other things, bring about the degradation of extensin (hydroxyproline-rich protein) present in wood, thus improving access to the lignin.

Other suitable protective colloids are amino acids, monosaccharides, oligosaccharides, polyethylene glycol [PEG] types of a wide range of molecular weights, polyethylene oxides, polyethyleneimines and polydimethylsiloxanes.

It is also possible to add to the enzyme component system of the invention substances capable of increasing the hydrophobicity of the reaction mixture, thus bringing about the swelling of the lignin and the fibers and which makes them more susceptible to attack. Such substances are, for example, glycols, such as propylene glycol and ethylene glycol, glycol ethers such as ethylene glycol dimethyl ether etc., and solvents, for example, alcohols such as methanol, ethanol, butanol, amyl alcohol, cyclohexanol, benzyl alcohol and chlorohydrin, phenols such as phenol, methylphenols and methoxyphenols, aldehydes such as formaldehyde and chloral, mercaptans such as butyl mercaptan, benzyl mercaptan and thioglycolic acid, organic acids such as formic, acetic and chloroacetic acid, amines such as ammonia and hydrazine, hydrotropic solvents, for example concentrated solutions of sodium benzoate, other substances such as benzenes, pyridines, dioxane, ethyl acetate, and other basic solvents such as OH<sup>-</sup>/H<sub>2</sub>O or OH<sup>-</sup>/alcohol etc..

The process according to the invention can be used not only for the delignification (bleaching) of sulfate, sulfite, organosolv or other wood pulps or lignins, but also for the preparation of wood pulp in general, whether from wood or annual plants, when it is desired to carry out the defibrillation by the usual cooking (digestion) process (possibly combined with mechanical processing or pressure), namely by very gentle digestion, up to kappa numbers in the range from about 50 - 120 kappa.

In the bleaching as in the preparation of wood pulps, the treatment with the enzyme component system (ECS) of the invention can be applied once or several times, either before and/or after the washing and extraction of the treated material with NaOH etc., or without these intermediate steps, but also before and/or after pretreatment or post-treatment steps, such as acid washing, Q-steps, alkaline leaching or bleaching steps such as peroxide bleaching, O<sub>2</sub>-enhanced peroxide steps, pressurized peroxide steps, O<sub>2</sub>-delignification, Cl<sub>2</sub>-bleaching, ClO<sub>2</sub>-bleaching, Cl<sub>2</sub>/ClO<sub>2</sub>-bleaching, peracid bleaching, peracid-enhanced O<sub>2</sub>/peroxide bleaching, ozone bleaching, dioxirane bleaching, reductive bleaching steps, other treatments such as swelling steps, sulfonations, NO/NO<sub>2</sub> treatments, nitrosylsulfuric acid treatment, enzyme treatments, for example treatments with hydrolases, such as cellulases and/or hemicellulases (for example, xylanase, mannase etc) and/or amylases and/or pectinases and/or proteinases and/or lipases and/or amidases and/or oxidoreductases such as, for example, laccases and/or peroxidases etc., or several combined treatments.

This results in substantially further reduced kappa values and substantially increased brightness. Before the ECS treatment, it is also possible to insert an O<sub>2</sub> step or, as already mentioned, carry out an acid wash or a Q-step (chelation step).

The invention will be further illustrated by way of the following examples:

## EXAMPLE 1

### Enzymatic Bleaching of O<sub>2</sub>-delignified Softwood (Sulfate Pulp)

5 g, absolutely dry basis, of wood pulp (O<sub>2</sub>-delignified softwood), pulp consistency 30% (about 17 g moist) was added to solutions prepared as follows:

A) To 20 mL of tap water were added 1 mg of tetradecanoic acid, 5 mg of benzophenone and 2.5 mg of H<sub>2</sub>O<sub>2</sub> (30%) with agitation. The pH was adjusted with sulfuric acid and/or sodium hydroxide solution so that, after addition of the wood pulp and the enzyme, the pH was 7.5.

B) To 5 mL of tap water was added 5 mg of lipase from *Humicola lanuginosa* (about 25,000 IU).

Solutions A and B were combined and diluted to 33 mL. After addition of the wood pulp, the material was mixed in a dough mixer for 2 minutes. The material was then transferred to a reaction vessel preheated to 45 °C and was allowed to incubate 1-4 hours under atmospheric pressure.

The material was washed over a nylon screen (30 µm) and extracted for one hour at 60 °C, 2% consistency and using 8% NaOH per gram of wood pulp. The material was again washed after which the kappa number was determined. For results see Table 1.

### EXAMPLE 1a

### Enzymatic Bleaching of O<sub>2</sub>-delignified Softwood (Sulfate Pulp)

5 g, absolutely dry basis, of wood pulp (O<sub>2</sub>-delignified softwood), pulp consistency 30% (about 17 g moist) was added to solutions prepared as follows:

A) To 20 mL of tap water were added 1 mg of tetradecanoic acid, 5 mg of acetone and 2.5 mg of H<sub>2</sub>O<sub>2</sub> (30%) with agitation. The pH was adjusted with sulfuric acid and/or sodium hydroxide solution so that, after addition of the wood pulp and the enzyme, the pH was 7.5.

B) To 5 mL of tap water was added 5 mg of lipase from *Humicola lanuginosa* (about 25,000 IU).

Solutions A and B were combined and diluted to 33 mL. After addition of the wood pulp, the material was mixed in a dough mixer for 2 minutes. The material was then

transferred to a reaction vessel preheated to 45 °C and was allowed to incubate 1-4 hours at atmospheric pressure.  
The material was washed over a nylon screen (30 µm) and extracted for one hour at 60 °C, 2% pulp consistency and using 8% NaOH per gram of wood pulp. The material was again washed, after which the kappa number was determined. For results see Table 1.

#### **EXAMPLE 1b (+ H<sub>2</sub>O<sub>2</sub>, with Nitrilamine as Activator)**

##### **Enzymatic Bleaching of O<sub>2</sub>-delignified Softwood (Sulfate Pulp)**

5 g, absolutely dry basis, of wood pulp (O<sub>2</sub>-delignified softwood), pulp consistency 30% (about 17 g moist) was added to solutions prepared as follows:

A) To 20 mL of tap water were added 1 mg of tetradecanoic acid, 5 mg of acetone, 2.5 mg of H<sub>2</sub>O<sub>2</sub> (30%) and 0.5 mg of nitrilamine with agitation. The pH was adjusted with sulfuric acid and/or sodium hydroxide solution so that, after addition of the wood pulp and the enzyme, the pH was 7.5.

B) To 5 mL of tap water was added 5 mg of lipase from *Humicola lanuginosa* (about 25,000 IU).

Solutions A and B were combined and diluted to 33 mL. After addition of the wood pulp, the material was mixed in a dough mixer for 2 minutes. The material was then transferred to a reaction vessel preheated to 45 °C and was allowed to incubate 1-4 hours at atmospheric pressure.

The material was washed over a nylon screen (30 µm) and extracted for one hour at 60 °C, 2 % pulp consistency and using 8% NaOH per gram of wood pulp. The material was again washed, after which the kappa number was determined. For results see Table 1.

#### **EXAMPLE 2**

##### **Enzymatic Bleaching of O<sub>2</sub>-delignified Hardwood (Sulfate Pulp)**

5 g, absolutely dry basis, of wood pulp (O<sub>2</sub>-delignified hardwood), pulp consistency 30% (about 17 g moist) was added to solutions prepared as follows:

A) To 20 mL of tap water were added 1 mg of tetradecanoic acid, 5 mg of benzophenone and 2.5 mg of H<sub>2</sub>O<sub>2</sub> (30%) with agitation. The pH was adjusted with sulfuric acid and/or sodium hydroxide solution so that, after addition of the wood pulp and the enzyme, the pH was 7.5.

B) To 5 mL of tap water was added 5 mg of lipase from *Humicola lanuginosa* (about 25,000 IU).



Solutions A and B were combined and diluted to 33 mL. After addition of the wood pulp, the material was mixed in a dough mixer for 2 minutes. The material was then transferred to a reaction vessel preheated to 45 °C and was allowed to incubate 1-4 hours at atmospheric pressure.

The material was washed over a nylon screen (30 µm) and extracted for one hour at 60 °C, 2% pulp consistency and using 8% NaOH per gram of wood pulp. The material was again washed after which the kappa number was determined. For results see Table 1.

### EXAMPLE 2a

5 g, absolutely dry basis, of wood pulp (O<sub>2</sub>-delignified hardwood), pulp consistency 30% (about 17 g moist) was added to solutions prepared as follows:

A) To 20 mL of tap water were added 1 mg of tetradecanoic acid, 5 mg of acetone and 2.5 mg of H<sub>2</sub>O<sub>2</sub> (30%) with agitation. The pH was adjusted with sulfuric acid and/or sodium hydroxide solution so that, after addition of the wood pulp and the enzyme, the pH was 7.5.

B) To 5 mL of tap water was added 5 mg of lipase from *Humicola lanuginosa* (about 25,000 IU).

Solutions A and B were combined and diluted to 33 mL. After addition of the wood pulp, the material was mixed in a dough mixer for 2 minutes. The material was then transferred to a reaction vessel preheated to 45 °C and was allowed to incubate 1-4 hours at atmospheric pressure.

The material was washed over a nylon screen (30 µm) and extracted for one hour at 60 °C, 2% pulp consistency and using 8% NaOH per gram of wood pulp. The material was again washed, after which the kappa number was determined. For results see Table 1.

### EXAMPLE 3

#### Enzymatic Bleaching of O<sub>2</sub>-delignified Softwood (Sulfate Pulp)

5 g, absolutely dry basis, of wood pulp (O<sub>2</sub>-delignified softwood), pulp consistency 30% (about 17 g moist) was added to solutions prepared as follows.

A) To 20 mL of tap water were added 1 mg of tetradecanoic acid, 5 mg of benzophenone and 2.5 mg of H<sub>2</sub>O<sub>2</sub> (30%) with agitation. The pH was adjusted with sulfuric acid and/or sodium hydroxide solution so that, after addition of the wood pulp and the enzyme, the pH was 7.5.

B) To 5 mL of tap water was added 200 IU of amidase from *Pseudomonas aeruginosa* (Sigma A 6691) (1 IU = conversion of 1 µmole of acetamide and hydroxylamine to acetohydroxamic acid and NH<sub>3</sub> per minute at pH 7.2 and 37 °C).

Solutions A and B were combined and diluted to 33 mL. After addition of the wood pulp, the material was mixed in a dough mixer for 2 minutes. The material was then transferred to a reaction vessel preheated to 45 °C and was allowed to incubate 1-4 hours at atmospheric pressure.

The material was washed over a nylon screen (30 µm) and extracted for one hour at 60 °C, 2% pulp consistency and using 8% NaOH per gram of wood pulp. The material was again washed, after which the kappa number was determined. For results see Table 1.

**TABLE 1**

Wood pulp	% Delignification (before extraction)	% Delignification (after extraction)
a) Softwood (untreated)	----	5.8%
b) Softwood (lipase-treated)	19% <u>17.5%</u>	32.0% <u>31%*</u> <u>35%**</u>
c) Hardwood (untreated)	----	6.5%
d) Hardwood (lipase-treated)	21% <u>18%*</u>	33% <u>28%*</u>
e) Softwood (amidase-treated)	15.5%	23%
f) Comparative example: laccase + HOBT 5 kg/ton of wood pulp, other conditions as in WO 96/18770 (pulp a/b)	17.5%	22%

\*Underlined values were obtained with acetone as the ketone.

\*\*Value obtained with added nitrilamine.

## **II) Use in Enzymatic Wastewater Treatment, for Example of Grinder Wastewater in the Paper Industry**

Because in this application the polymerization of lignin or lignin constituents contained in the wastewater is desired rather than lignin degradation, the enzyme component system (ECS) of the invention is used with a small amount of added polymerization catalyst.

One component of the enzyme component system (ECS) of the invention used is an enzyme, preferably lipase from *Aspergillus spec.*, at a concentration from 0.05 to 50 mg per liter of wastewater and preferably from 0.05 to 10 mg of enzyme per liter of wastewater (corresponding to about 250 to 50,000 IU per liter of wastewater) (1 IU hydrolyzes 1  $\mu$  equivalent of triglyceride fatty acid in 1 hour at pH 7.7 and 37 °C).

The treatment of the grinder wastewater with the enzyme component system of the invention is preferably carried out in the presence of oxygen or air at atmospheric pressure or slight positive oxygen pressure and at a pH from 2 to 11 and preferably from 3 to 6, at a temperature from 20 to 95 °C and preferably from 40 to 95 °C.

Another component is the oxidant, preferably H<sub>2</sub>O<sub>2</sub>, which is used at a concentration from 0.05 to 200 mg per liter of wastewater (100% basis) and preferably from 0.05 to 50 mg per liter of wastewater.

Another component consists of one or more fatty acids, preferably C<sub>6</sub> to C<sub>26</sub> and particularly C<sub>6</sub> to C<sub>16</sub>, fatty acids, and more particularly tetradecanoic or dodecanoic acid at a concentration from 0.05 to 200 mg per liter, and preferably at a concentration from 0.05 to 10 mg per liter, of wastewater.

Another component is a ketone, preferably, for example, benzophenone at a concentration from 0.05 to 200 mg per liter of wastewater and preferably at a concentration from 0.05 to 50 mg per liter of wastewater.

Moreover, to increase the efficiency of the process and to use less precipitant (mostly sodium aluminate/aluminum sulfate) which represents the main cost factor, a polymerization catalyst is used, mostly a phenolic substance or a polycyclic compound with several oxidizable hydroxyl groups, in our case preferably, for example, purpurogallin.

These substances are used at a concentration from 0.005 to 200 mg per liter of wastewater and preferably at a concentration from 0.005 to 50 mg per liter of wastewater.

The invention will be further illustrated by way of the following examples:

#### EXAMPLE 4

190 mL of grinder wastewater was adjusted to pH 6, its temperature was adjusted to 45 °C in an appropriate jacketed reaction vessel and to it were added the following solutions:

- 1) Enzyme solution: lipase (*Aspergillus spee.*): 1 mg in 0.1 mL of water
- 2) Fatty acid solution: 1 mg of dodecanoic acid in 1 mL of water
- 3) Ketone solution: 1 mg of 2,2',4,4'-tetrahydroxybenzophenone in 1 mL of water
- 4) Polymerization catalyst: 0.1 mg of purpurogallin in 0.1 mL of water.

The reaction was initiated by addition of solution 5) (oxidant:  $\text{H}_2\text{O}_2$ ), namely of a solution of 3.3 mg of  $\text{H}_2\text{O}_2$  (30%) in 0.1 mL of water, and the volume was adjusted to 200 mL with preheated water. The reaction was allowed to proceed for 1 to 4 hours and preferably for 2 hours. The wastewater was then either only filtered or filtered and precipitated with 0.2%/0.2% or 0.5%/0.5% aluminum sulfate solution/sodium aluminate solution, 10 wt % in each case and based on the initial value without treatment. The lignin which in untreated grinder wastewater is usually present in an amount from 600 to 900 mg per liter was determined quantitatively by photometry at 280 nm. The drop in lignin is a measure of COD reduction and of the efficiency of the system.

The results are collected in Table 2.

#### EXAMPLE 5 (without polymerization catalyst)

190 mL of grinder wastewater was adjusted to pH 6, its temperature was adjusted to 45 °C in an appropriate jacketed reaction vessel and to it were added the following solutions:

- 1) Enzyme solution: lipase (*Aspergillus spec.*): 1 mg in 0.1 mL of water
- 2) Fatty acid solution: 1 mg of dodecanoic acid in 1 mL of water
- 3) Ketone solution: 1 mg of 2,2',4,4'-tetrahydroxybenzophenone in 1 mL of water

The reaction was initiated by addition of solution 4) (oxidant-  $\text{H}_2\text{O}_2$ ), namely a solution of 3.3 mg of  $\text{H}_2\text{O}_2$  (30%) in 0.1 mL of water, and the volume was adjusted to 200 mL with preheated water. The reaction was allowed to proceed for 1 to 4 hours and preferably for 2 hours. The wastewater was then either only filtered or filtered and precipitated with 0.2%/0.2% or 0.5%/0.5% aluminum sulfate solution/sodium aluminate solution, 10 wt % in each case based on the initial value without treatment. The lignin which in untreated grinder wastewater is usually present in an amount from 600 to 900 mg per liter was determined quantitatively by photometry at 280 nm. The drop in lignin is a measure of COD reduction and of the efficiency of the system.

The results are collected in Table 2.

**TABLE 2**

**Treatment** **Residual Lignin After 2 Hours**  
(0 Value = 600 mg/liter)

	COD (mg/L)
No treatment (filtered)	800
No treatment (filtered/precipitated*)	720
With treatment (lipase) (filtered/precipitated), (with polymerization cat.)**	100
Comparative treatment: with 25,000 IU of laccase per liter of wastewater (filtered/precipitated*) (with polym. catalyst)**	170
With treatment (lipase) (filtered/precipitated*) (without polym. catalyst)	220
With treatment (filtered/precipitated*) with polym. catalyst (Humicola lipase) (For the test with polymerization and/or modification of lignin, see below)	160

\* Only the precipitations at 0.5%/0.5% are shown.

\*\* Polymerization catalyst

### III) Use in the Preparation of Lignin Solutions or Gels, Corresponding Binders/Adhesives and of Wood-Based Composites

In this application, too, the polymerization of the lignin or lignin-containing materials is desired and not lignin degradation. Hence, the enzyme component system (ECS) of the invention is used with a small amount of added polymerization catalyst.

Because it was found that the polymerization of lignin, for example in groundwood pulp wastewater (grinder wastewater) is also a good system for evaluating general polymerization properties for the use of the enzyme component system (ECS) in the preparation of lignin solutions or gels, of the corresponding binders/adhesives and of wood-based composites, tests were carried out with the same experimental formulations as for the wastewater.

In this regard, it is known from the cited patents WO 94/01488, WO 93/25622, WO 93/23477 and DE 3 037 992 C2 that, for example, in the production of particle board the binder made by polymerization and dissolution of lignin is applied by spraying it onto the wood fiber material in an amount of about 40 to 100 g per kg of said material which is then subjected to pressing at a pressure of about 20-40 kg / cm<sup>2</sup> for about 2-4 min and at a temperature rising from about 35 to 190 °C within about 20 seconds. The pressures and temperatures used for pressing can, of course, also be substantially lower, and subsequent curing of the binder/wood fiber mixture by continuing enzyme-catalyzed reactions may be desired.

To evaluate the polymerization properties of ECS for this application, we used as the model system, as stated hereinabove, the above-described system for removing lignin from grinder wastewater.

As one component of the enzyme component system (ECS) of the invention was used an enzyme, preferably lipase from *Humicola lanuginosa*, at a concentration from 0.05 to 50 mg per liter of wastewater and preferably from 0.05 to 10 mg of enzyme per liter of wastewater (corresponding to about 250 to 50,000 IU per liter of wastewater) (1 IU hydrolyzes 1  $\mu$  equivalent of triglyceride fatty acid in 1 hour at pH 7.7 and 37 °C). The treatment of the grinder wastewater with the enzyme component system of the invention is preferably carried out in the presence of oxygen or air at atmospheric pressure or slight positive oxygen pressure and at a pH from 2 to 11 and preferably from 3 to 6, at a temperature from 20 to 95 °C and, preferably from 40 to 95 °C. Another component is the oxidant, preferably H<sub>2</sub>O<sub>2</sub> which is used at a concentration from 0.05 to 200 mg per liter of wastewater (100% basis) and preferably from 0.05 to 50 mg/liter of wastewater.

Another component consists of one or more fatty acids, preferably a C<sub>6</sub> to C<sub>26</sub>, particularly C<sub>6</sub> to C<sub>16</sub> fatty acid and more particularly tetradecanoic or dodecanoic acid at a concentration from 0.05 to 200 mg/liter of wastewater and preferably at a concentration from 0.05 to 50 mg/liter of wastewater.

Another component is a ketone, preferably, for example, benzophenone at a concentration from 0.05 to 200 mg/liter of wastewater and preferably at a concentration from 0.05 to 50 mg/liter of wastewater.

Moreover, as already stated in the foregoing, to increase the efficiency of the process a polymerization catalyst is used, mostly a phenolic substance or a polycyclic compound with several oxidizable hydroxyl groups, in our case preferably, for example, purpurogallin.

These substances are used at a concentration from 0.005 to 200 mg per liter of wastewater and preferably at a concentration from 0.005 to 50 mg per liter of wastewater.

#### EXAMPLE 6

190 mL of grinder wastewater was adjusted to pH 8.5, preheated to a temperature of 45 °C in an appropriate jacketed reaction vessel and to it were added the following solutions:

- 1) Enzyme solution: lipase (*Humicola lanuginosa*): 1 mg in 0.1 mL of water
- 2) Fatty acid solution: 1 mg of dodecanoic acid in 1 mL of water
- 3) Ketone solution: 1 mg of 2,2',4,4'-tetrahydroxybenzophenone in 1 mL of water
- 4) Polymerization catalyst: 0.1 mg of purpurogallin in 0.1 mL of water.

The reaction was initiated by addition of solution 5) (oxidant:  $H_2O_2$ ), namely a solution of 3.3 mg of  $H_2O_2$  (30%) in 0.1 mL of water, and the volume was adjusted to 200 mL with preheated water. The reaction was allowed to proceed for 1 to 4 hours and preferably for 2 hours. The wastewater was then either only filtered or filtered and precipitated with 0.2%/0.2% or 0.5%/0.5% aluminum sulfate solution/sodium aluminate solution, 10 wt % in each case, based on the initial value without treatment. The lignin which in untreated grinder wastewater is usually present in an amount from 600 to 900 mg per liter was determined quantitatively by photometry at 280 nm. The drop in lignin is a measure of COD reduction and of the efficiency of the system.

The results are collected in Table 2.

#### IV) Use as Enzymatic Deinking System

In this application, no lignin degradation is desired but, rather, a swelling effect on the lignin-containing fibers so as to bring about the detachment of adhering printing ink particles, an effect similar to that of sodium hydroxide solution in conventional chemical deinking.

To the enzyme component system (ECS) are added besides the usual components such as lipase, oxidant, fatty acid and ketone, also a number of phenolic substances which serve as polymerization catalysts in wastewater treatment and in lignin polymerization/modification applications. Here, we found, surprisingly, that these substances cause a shift in the pH for the optimum enzyme activity thus improving performance.

Also surprisingly, we found that the addition of a reducing agent, preferably dithionite or bisulfite increases the efficiency of ink detachment.

One component of the enzyme component system (ECS) of the invention used is an enzyme, preferably lipase from *Humicola lanuginosa*, at a concentration from 5 to 500 mg per kg of air-dried waste paper, and preferably from 5 to 100 mg of enzyme per kg of waste paper (corresponding to about 25,000 to 500,000 IU per kg of waste paper) (1 IU hydrolyzes 1  $\mu$  equivalent of triglyceride fatty acid in 1 hour at pH 7.7 and 37 °C). The treatment of the waste paper with the enzyme component system of the invention for the purpose of removing printing ink particles is preferably carried out in the presence of oxygen or air at atmospheric pressure or slightly positive oxygen

pressure (maximum 2 bar) and at a pH from 7 to 11 and preferably 7 to 9, at a temperature from 20 to 95 °C and preferably from 40 to 95 °C.

Another component is the oxidant, preferably H<sub>2</sub>O<sub>2</sub>, which is used at a concentration from 5 to 5000 mg per kg of waste paper (100% basis) and preferably from 5 to 1000 mg per kg of waste paper.

Another component consists of one or more fatty acids, preferably C<sub>6</sub> to C<sub>26</sub>, and particularly C<sub>6</sub> to C<sub>16</sub>, fatty acids, and more particularly tetradecanoic or dodecanoic acid at a concentration from 5 to 2000 mg per kg of waste paper and preferably at a concentration from 5 to 500 mg per kg of waste paper.

Another component is a ketone, preferably, for example, benzophenone at a concentration from 5 to 2000 mg per kg of waste paper and preferably at a concentration from 5 to 500 mg per kg of waste paper.

Moreover, to increase the efficiency of the process, the abovesaid compounds are used, for example phenolic substances or polycyclic compounds with several oxidizable hydroxyl groups and preferably, for example, bisphenol A. These substances are employed at a concentration from 1 to 2000 mg per kg of waste paper and preferably at a concentration from 1 to 500 mg per kg of waste paper.

In addition, a reducing agent is used, preferably Na-dithionate or Na-bisulfite, at a concentration from 0. 1 to 1000 mg per kg of waste paper and preferably at a concentration from 0. 1 to 200 mg per kg of waste paper.

To collect the printing ink particles, commercial detergents are used as collectors, preferably Incopur brands, for example Incopur RSGA, at a concentration from 1 to 5000 mg per kg of waste paper, and preferably from 1 to 1000 mg per kg of waste paper.

To enhance the detaching effect on many waste paper compositions, additional enzymes can be added, for example cellulases and/or hemicellulases (for example, xylanase and/or mannanase etc.) and/or pectinases and/or oxidoreductases.

The invention will be further illustrated by way of the following examples:

#### EXAMPLE 7

About 10 kg of water (preheated to about 45 °C) was added to the pulper of a Lamort laboratory deinking apparatus, and the pH was adjusted with sodium hydroxide solution (and/or sulfuric acid) so that after the addition of 1.5 kg of air-dried waste paper (50% newspapers, 50% magazines) which had been cut into about 2 x 3 cm pieces and after the addition of the other system constituents the pH was 8.0 to 8.5.

These system constituents were (per kg of air-dried waste paper):

- a) 500,000 IU of lipase from *Humicola lanuginosa* per 100 mL of tap water
- b) 0.1 g of dodecanoic acid per 100 mL of tap water
- c) 0.1 g of benzophenone per 100 mL of tap water
- d) 0.1 g of bisphenol A per 20 mL of 0. 1 molar NaOH
- e) 0.02 g of Na bisulfite per 10 mL of tap water
- f) 0.5 g of Incopur RSGA per 100 mL of tap water
- g) 1 g of H<sub>2</sub>O<sub>2</sub> (30%) per 100 mL of tap water (added at the end).

The pulper was started after the addition of system constituents a) to g) and during the addition of the waste paper. The total quantity of water was then adjusted to 15 kg with approximately 45 °C tap water. The pulping process was allowed to proceed for 10



minutes. For further reaction, the fiber suspension was transferred to a heated vessel where it was allowed to incubate at about 40–45 °C for 15 to 45 minutes. In a Voith flotation cell, 100 g of pulp, absolutely dry basis, (after this incubation) was adjusted to a total volume of about 20 L with tap water (45 °C) and subjected to flotation for 10 to 20 minutes. The accepted stock was discharged, test sheets were prepared from it in a commercial sheet former, dried and the ISO brightness were determined. Table 3 shows the results.

(ISO = International Standardization Organization)

### EXAMPLE 8

The method was the same as in Example 7, but instead of the enzyme component system (ECS) of the invention only water was used. Table 3 shows the results.

### EXAMPLE 9

About 1 kg of water (preheated to about 45 °C) was added to a dough mixer, and the pH was adjusted with sodium hydroxide solution (and/or sulfuric acid) so that after the addition of 150 g of air-dried waste paper (50% newspapers, 50% magazines) which had been cut into about 2 x 3 cm pieces and after the addition of the other system constituents the pH was 8.0 to 8.5.

These system constituents were (per 100 g of air-dried waste paper):

a) 5000 IU of amidase from *Pseudomonas aeruginosa* (Sigma A 6691) per 100 mL of tap water

(1 IU = conversion of 1  $\mu$ mol of acetamide and hydroxylamine to acetohydroxamic acid and  $\text{NH}_3$  per minute at pH 7.2 and 37 °C)

b) 0.01 g of dodecanoic acid per 100 mL of tap water

c) 0.01 g of benzophenone per 100 mL of tap water

d) 0.01 g of bisphenol A per 20 mL of 0.1 molar NaOH

e) 0.002 g of Na bisulfite per 10 mL of tap water

f) 0.05 g of Incopur RSGA per 100 mL of tap water

g) 0.1 g of  $\text{H}_2\text{O}_2$  (30%) per 100 mL of tap water (added at the end).

The dough mixer was started after the addition of system constituents a) to g) and during the addition of the waste paper. The total quantity of water was then adjusted to 1.5 kg with tap water (45 °C). The pulping process was allowed to proceed for 10 minutes.

For further reaction, the fiber suspension was transferred to a heated vessel where it was allowed to incubate at about 40–45 °C for 15 to 45 minutes.

In a Voith flotation cell, 100 g of pulp, absolutely dry basis, (after this incubation) was adjusted to a total volume of about 20 L with tap water (45 °C) and subjected to flotation for 10 to 20 minutes. The accepted stock was discharged, test sheets were prepared from it in a commercial sheet former, dried and the ISO brightness and were determined. Table 3 shows the results.

### EXAMPLE 10 (Chemical System)

About 10 kg of water (preheated to about 45 °C) was added to the pulper of a Lamort laboratory deinking apparatus, and 1.5 kg of air-dried waste paper (50% newspapers, 50% magazines), cut into approximately 2 x 3 cm pieces, was added after the addition of the following chemicals (based on air-dried pulp):

- 1) 0.8 wt % of soap (DR 3, Henkel)
- 2) 3.5% of water glass
- 3) 2% of sodium hydroxide (100%)
- 4) 1 % H<sub>2</sub>O<sub>2</sub> (100%)

The pulper was started during waste paper addition. The total quantity of water was then adjusted to 15 kg with about 45 °C tap water. The pulping process was allowed to proceed for 10 minutes.

For further reaction, the fiber suspension was transferred to a heated vessel where it was allowed to incubate at about 40-45 °C for 15 to 45 minutes,

In a Voith flotation cell, 100 g of pulp, absolutely dry basis, (after this incubation) was adjusted to a total volume of about 20 L with tap water (45 °C) and subjected to flotation for 10 to 20 minutes. The accepted stock was discharged, test sheets were prepared from it in a commercial sheet former, dried and the ISO brightness were determined. Table 3 shows the results.

TABLE 3

System	ISO Brightness, %
Water only	51
Chemical system	61
ECS/lipase	58.5
ECS/amidase	57
Comparative system: laccase (800,000 IU/kg of waste paper + bisphenol A + Na bisulfite (0.1 or 0.02 g/kg of waste paper), for other con- ditions see WO 91/14820; WO 92/20857	55.5

### V) Use as Oxidation System in Organic Synthesis

From the multiplicity of possible uses of the enzyme component system (ECS) of the invention, such as in hydroxylation reactions, oxidation of unsaturated aliphatics, Baeyer-Villiger oxidations, oxidation of heterocycles, carbon-carbon dehydrogenations

and other oxidation reactions, by way of the following examples, the oxidation of alcohols to aldehydes and of aromatic methyl groups to aldehydes are described.

It is known from the literature that these reactions can be carried out with the oxidoreductase laccase and a mediator such as ABTS (2,2'-azino-bis(3-ethylbenzothiazolin-6-sulfonic acid), see T. Rosenau et al., Synthetic Communications 26 (2), 315-320 (1996) and A Potthast et al., J. Org. Chem. (60), pp. 4320-4321 (1995). The main advantage of the process of the invention over these processes is its lower cost and better performance, particularly based on the cost.

One of the components of the enzyme component system (ECS) of the invention is an enzyme, preferably lipase from, for example, *Humicola lanuginosa*, used at a concentration of 0.05 to 5 mg per 10 mmoles of substrate, preferably from 0.05 to 3 mg per 10 mmoles of substrate (which corresponds to about 250 to 15,000 IU) (1 IU hydrolyzes 1  $\mu$  equivalent of a triglyceride fatty acid in 1 hour at pH 7.7 and 37 °C). Preferably, the oxidation reaction with the enzyme component system of the invention is carried out in the presence of oxygen or air at atmospheric pressure or a slightly positive O<sub>2</sub> pressure and at pH from 2 to 11, preferably at pH 3-9, at a temperature of 20 to 95 °C, preferably 40-95 °C and a 5 to 100 mmolar, preferably 5 to 50 mmolar, substrate concentration.

Another component is the oxidant, preferably H<sub>2</sub>O<sub>2</sub> (100%), which is used at a concentration from 0.05 to 100 mg per 10 mmoles of substrate and preferably from 0.05 to 30 mg per 10 mmoles of substrate.

Another component consists of one or more fatty acids, preferably C<sub>6</sub> to C<sub>26</sub>, and particularly C<sub>6</sub> to C<sub>16</sub> fatty acids, and more particularly tetradecanoic or dodecanoic acid at a concentration from 0.05 to 100 mg per 10 mmoles of substrate and preferably at a concentration from 0.05 to 30 mg per 10 mmoles of substrate.

Another component is a ketone, preferably, for example, benzophenone at a concentration from 0.05 to 100 mg per 10 mmoles of substrate and preferably at a concentration from 0.05 to 30 mg per 10 mmoles of substrate.

The invention will be further illustrated by way of the following examples:

#### EXAMPLE 11 (Oxidation of Benzyl Alcohols to Aldehydes)

The following components were added to 50 mL of 0.1 molar pH 4.5 acetate buffer in a 250-mL reaction flask:

- 1) p-methoxybenzyl alcohol in 30 mL of tetrahydrofuran [THF] (20-molar in total volume)
- 2) 2 mg of lipase from *Humicola lanuginosa*
- 3) 5 mg of dodecanoic acid
- 4) 25 mg of benzophenone

The reaction was started by addition of 12.5 mg of H<sub>2</sub>O<sub>2</sub> (30%) and was allowed to proceed for 12 to 24 hours. Then, 0.5 mL of the reaction solution was removed, extracted with CH<sub>2</sub>Cl<sub>2</sub>, and the p-methoxybenzaldehyde content was determined by GC or GC-MS. The results are shown in Table 4.

**EXAMPLE 12 (Oxidation of Aromatic Methyl Groups to Aldehydes)**

The following components were added to 50 mL of 0.1 molar pH 4.5 acetate buffer in a 250-mL reaction flask:

- 1) toluene in 30 mL of THF (20-molar in total volume)
- 2) 2 mg of lipase from *Humicola lanuginosa*
- 3) 5 mg of dodecanoic acid
- 4) 25 mg of benzophenone

The reaction was started by addition of 12.5 mg of  $\text{H}_2\text{O}_2$  (30%) and was allowed to proceed for 12 to 24 hours. Then, 0.5 mL of the reaction solution was removed, extracted with  $\text{CH}_2\text{Cl}_2$ , and the benzaldehyde content was determined by GC or GC-MS. The results are shown in Table 4.

**TABLE 4**

Substrate	Oxidized Substrate	Conversion, %
P-Methoxybenzyl alcohol (lipase)	p-methoxybenzaldehyde	98
p-Methoxybenzyl alcohol (ABTS/laccase)	p-methoxybenzaldehyde	90
Toluene (lipase)	benzaldehyde	98
Toluene (ABTS/laccase)	benzaldehyde	92

**VI) Use in Coal Liquefaction**

Recently, the use of white rotting fungi was studied in the liquefaction of lignite and anthracite, and the general feasibility thereof was confirmed. Patent applications WO 94/29510 and WO 96/18770 have also disclosed the general feasibility of using fungus-free systems based on oxido-reductases and special mediators.

Surprisingly, we were able to confirm for the enzyme component system (ECS) of the invention that it, too, can be used for "liquefying" the lignin-like tridimensional network of polycyclic aromatic ring systems of lignite and anthracite.

One of the components of the enzyme component system (ECS) of the invention is an enzyme, preferably lipase from, for example, *Humicola lanuginosa*, used at a concentration of 0.05 to 20 mg per gram of ground lignite, absolutely dry basis, preferably from 0.05 to 10 mg per gram of coal (corresponding to about 250 to 50,000 IU) (1 IU hydrolyzes 1  $\mu$  equivalent of a triglyceride fatty acid in 1 hour at pH 7.7 and 37 °C).

Preferably, the coal treatment with the enzyme component system of the invention is carried out in the presence of oxygen or air at atmospheric pressure or at a slightly positive  $O_2$  pressure and at pH from 2 to 11, preferably at pH 3-9, at a temperature of 20 to 95 °C, preferably 40-95 °C, and a coal consistency of 0.5 to 40%.

An unusual and surprising finding concerning the use of enzymes is that when the enzyme component system of the invention is employed, the consistency of the material can be increased and the performance is markedly improved. For economic reasons, the process according to the invention is carried out at a coal consistency from 4 to 35% and particularly from 4 to 15%.

Another component is the oxidant, preferably  $H_2O_2$ , which is used at a concentration from 0.05 to 100 mg per gram of coal (100% basis) and preferably from 0.05 to 50 mg per gram of coal.

Another component consists of one or more fatty acids, preferably  $C_6$  to  $C_{26}$ , particularly  $C_6$  to  $C_{16}$  fatty acids, and more particularly tetradecanoic or dodecanoic acid at a concentration from 0.05 to 100 mg per gram of coal and preferably at a concentration from 0.05 to 50 mg per gram of coal.

Another component is a ketone, preferably, for example, benzophenone at a concentration from 0.05 to 100 mg per gram of coal and preferably at a concentration from 0.05 to 50 mg per gram of coal.

The invention will be further illustrated by way of the following example:

### EXAMPLE 13

#### Enzymatic Coal Liquefaction

5 g of lignite or anthracite, absolutely dry basis, (particle size about 200 to 500,  $\mu$ ) was added to solutions prepared as follows:

A) To 20 mL of tap water were added 5 mg of tetradecanoic acid, 25 mg of benzophenone and 12.5 mg of  $H_2O_2$  (30%) per gram of coal with agitation. The pH was adjusted with sulfuric acid and/or sodium hydroxide solution so that, after addition of the coal and the enzyme, the pH was 8.

B) To 5 mL of tap water was added 10 mg of lipase from *Humicola lanuginosa* (about 50,000 IU).

Solutions A and B were combined and diluted to 45 mL. After addition of the coal, the material was mixed for 2 minutes. The material was then transferred to a reaction vessel preheated to 45 °C and allowed to incubate 1-4 hours.

The resulting coal of modified consistency was then removed from the reaction flask.

#### VII) Use as Bleaching Agent in Detergents

In using the enzyme component System (ECS) of the invention as a bleaching agent in detergents, one of the components is an enzyme, preferably lipase from, for example, *Humicola lanuginosa*, used at a concentration from 0.05 to 20 mg per 100 mL of washing solution and preferably from 0.05 to 10 mg of enzyme per 100 mL of washing solution (which corresponds to about 250 to 100,000 IU per 100 mL of washing solution) (1 IU hydrolyzes 1  $\mu$  equivalent of a triglyceride fatty acid in 1 hour at pH 7.7 and 37 °C).

Preferably, the bleaching with the enzyme component system of the invention is carried out in the presence of oxygen or air at atmospheric pressure or at a slightly positive  $O_2$  pressure and at pH from 2 to 12, preferably at pH 3-10, at a temperature of 20 to 95 °C and preferably 30-95 °C.

Another component is the oxidant, preferably  $H_2O_2$ , which is used at a concentration from 0.05 to 50 mg per 100 mL of washing solution (100% basis) and preferably from 0.05 to 20 mg per 100 mL of washing solution.

Another component consists of one or more fatty acids, preferably a  $C_6$  to  $C_{26}$ , particularly a  $C_6$  to  $C_{16}$  fatty acid and more particularly tetradecanoic or dodecanoic acid, at a concentration from 0.05 to 50 mg per 100 mL of washing solution and preferably at a concentration from 0.05 to 20 mg per 100 mL of washing solution.

Another component is a ketone, preferably, for example, benzophenone at a concentration from 0.05 to 50 mg per 100 mL of washing solution and preferably at a concentration from 0.05 to 20 mg per 100 mL of washing solution.

### Other Components

The bleaching system can also contain phenolic and/or nonphenolic compounds with one or more benzene rings. The following oxidants besides those mentioned hereinabove are particularly preferred: air, oxygen,  $H_2O_2$ , organic peroxides, sodium perborate and/or sodium percarbonate. Oxygen can also be generated in situ by  $H_2O_2 +$  catalase or the like, or  $H_2O_2$  can be generated in situ by  $GOD +$  glucose or similar systems.

Also preferred are multicomponent bleaching systems containing cation-generating metals salts. The cations  $Fe^{2+}$ ,  $Fe^{3+}$ ,  $Mn^{2+}$ ,  $Mn^{3+}$ ,  $Mn^{4+}$ ,  $Cu^{1+}$ ,  $Cu^{2+}$ ,  $Ti^{3+}$ ,  $Ce^{4+}$ ,  $Mg^{2+}$  and  $Al^{3+}$  are preferably used.

The bleaching system can also contain polysaccharides and/or proteins. Suitable polysaccharides are, glucans, mannans, dextrans, levans, pectins, alginates, vegetable gums and/or polysaccharides formed by fungi or produced in mixed cultures with yeasts. Suitable proteins are gelatins and albumins, among others. Also suitable are monosaccharides, oligosaccharides, amino acids, PEG, polyethylene oxides, polyethyleneimines and polydimethylsiloxanes.

### Use of the Multicomponent System

The multicomponent system can be used in combination with surface-active detergent constituents or detergent additives, which in themselves are known.

The invention will be further illustrated by way of the following examples:

### EXAMPLE 14

#### Effect of ECS on Tea-Stained Standard Cotton Fabrics

A (5x5 cm) piece of fabric was allowed to incubate in 100 mL of washing solution (in a 300-mL Erlenmeyer flask) at 40 °C for 40 min with reciprocating shaking (120 rpm). Before the beginning of incubation, the washing solution was subjected to a 10-min temperature equilibration period.

The washing solution was prepared with standard tap water (STW) at 14° dH'. The following enzyme doses were used: 2.5 mg of lipase from *Humicola lanuginosa*/100 mL, 2.5 mg of tetradecanoic acid/100 mL, 12.5 mg of benzophenone/100 mL and 6.5 mg of H<sub>2</sub>O<sub>2</sub> (30%). After decanting the washing liquor", cold water was added 3x in the form of a strong water jet and then decanted. The results are shown in Table 5.

## EXAMPLE 15

### Effect of ECS (Amidase) on Tea-Stained Standard Cotton Fabrics

A (5x5 cm) piece of fabric was allowed to incubate in 100 mL of washing solution (in a 300-mL Erlenmeyer flask) at 40 °C for 40 min with reciprocating shaking (120 rpm). Before the beginning of incubation, the washing solution was subjected to a 10-min temperature equilibration period.

The washing solution was prepared with standard tap water (STW) at 14° dH. The following doses were used: 1000 IU of amidase/ 100 mL (1 IU = conversion of 1 µmole of acetamide and hydroxylamine to acetohydroxamic acid per minute at pH 7.2 and 37 °C), 2.5 mg of tetradecanoic acid/1 00 mL, 12.5 mg of benzophenone/100 mL and 6.5 mg of H<sub>2</sub>O<sub>2</sub> (30%). After decanting the "washing liquor", cold water was added 3 x in the form of a strong water jet and then decanted.

The results are shown in Table 5.

(dH = one degree of German water hardness = 10 mg of CaO/L)

TABLE 5

	pH	Whiteness	Brightness
STW zero value	4.5	2.55	2.3
Heavy-duty detergent	10.1	8.9	6.15
STW + ECS (lipase)	8.5	7.5	7.2
STW + ECS (amidase)	8	6.9	6.3
Liquid detergent + ECS (lipase)	8.5	8.5	8.0
Comparative test:			
Liquid detergent + laccase + HOBT (conditions as in PCT/EP 96/02658;			
PCT/EP 94101967	5	6.5	6.0

### VIII) Use in the Bleaching/Decolorizing of Textiles

One of the components of the enzyme component system (ECS) of the invention used in the bleaching/decolorizing of textile fabrics is an enzyme, preferably lipase from, for example, *Humicola lanuginosa*, employed at a concentration of 0.05 to 10 mg per gram of denim (corresponding to about 250 to 25,000 IU per gram of denim) (1 IU hydrolyzes 1  $\mu$  equivalent of a triglyceride fatty acid in 1 hour at pH 7.7 and 37 °C). Preferably, the bleaching/decolorizing with the enzyme component system of the invention is carried out in the presence of oxygen or air at atmospheric pressure or at a slightly positive O<sub>2</sub> pressure and at pH from 2 to 11, preferably at pH 3-9, at a temperature of 20 to 95 °C, preferably 40-95 °C and a fabric density of 0.5 to 40% [fabric density = ratio of weight of fabric to weight of solution]

An unusual and surprising finding concerning the use of enzymes is that when the enzyme component system of the invention is used, the fabric density can be increased and the performance markedly improved. For economic reasons, the process according to the invention is carried out at a fabric density from 4 to 35% and particularly from 4 to 15%.

Another component is the oxidant, preferably H<sub>2</sub>O<sub>2</sub>, which is used at a concentration from 0.05 to 20 mg per gram of denim (100% basis) and preferably from 0.05 to 10 mg per gram of denim.

Another component consists of one or more fatty acids, preferably C<sub>6</sub> to C<sub>26</sub> and particularly C<sub>6</sub> to C<sub>16</sub> fatty acids, and more particularly tetradecanoic or dodecanoic acid at a concentration from 0.05 to 20 mg/g of denim and preferably at a concentration from 0.05 to 10 mg per gram of denim.

Another component is a ketone, preferably, for example, benzophenone, used at a concentration from 0.05 to 20 mg/g of denim and preferably at a concentration from 0.05 to 10 mg/g of denim.

The invention will be further illustrated by way of the following examples:

#### EXAMPLE 16

##### Bleaching with ECS + Lipase

1 g of denim fabric was placed in a 200-mL Erlenmeyer flask (fabric density 2%). The pH of the solution (tap water), the volume of which was 50 mL after the addition of all components, was preadjusted to pH 6 with 0.5 N H<sub>2</sub>SO<sub>4</sub>.

1 mg of lipase from *Humicola lanuginosa*, 0.5 mg of tetradecanoic acid, 1 mg of benzophenone and 2.5 mg of H<sub>2</sub>O<sub>2</sub> (30%) were added per gram of denim. The experiment was carried out in a shaking water bath (200 rpm) at 45 °C and a reaction time of 45 minutes. The piece of fabric was washed with tap water and dried in air. The brightness was then determined with an Elrepho instrument. The values obtained are given in Table 6.



## EXAMPLE 17

### Bleaching with ECS + Amidase

1 g of denim fabric was placed in a 200-mL Erlenmeyer flask (fabric density 2%). The pH of the solution (tap water), the volume of which was 50 mL after the addition of all components, was preadjusted to pH 6 with 0.5 N  $\text{H}_2\text{SO}_4$ . 200 IU of amidase from *Pseudomonas aeruginosa* (Sigma A 6691), 0.5 mg of tetradecanoic acid, 1 mg of benzophenone and 2.5 mg of  $\text{H}_2\text{O}_2$  (30%) were added per gram of denim. The experiment was carried out in a shaking water bath (200 rpm) at 45 °C and a reaction time of 45 minutes. The piece of fabric was washed with tap water and dried in air. The brightness was then determined with an Elrepho instrument. The values obtained are given in Table 6.

TABLE 6

System	pH	ISO Brightness
Untreated specimen	--	4.5
Laccase + violuric acid (comparative system)	3.5	13.5
ECS system + lipase	6.0	16.9
ECS system + amidase	6.0	14.5
Hypochlorite	4.5	n.d.

### Addition of Other Substances to the Enzyme Component System (ECS)

For all applications, the components of the enzymatic oxidation systems with enzyme action-enhancing compounds disclosed in DE 198 21 263.1, DE 198 20 947.9 and PCT/DE 98/01313 can be added to the enzyme component system (ECS) of the invention, such systems containing the following:

a) At least one oxidation catalyst, preferably enzymes such as oxidoreductases of classes 1.1.1. to 1.97 according to the International Enzyme Nomenclature: Committee of the International Union of Biochemistry and Molecular Biology (Enzyme Nomenclature, Academic Press, Inc., 1992, pp. 24-154) among which the following are particularly preferred: cellobiose: oxygen-1-oxidoreductase (cellobiose reductase) **1.1.3.25**, cellobiose: quinone-1-oxidoreductase **1.1.5.1**, bilirubin oxidase **1.3.3.5**, cytochrome oxidase **1.9.3**, oxygenases, lipooxygenases **1.13**, **1.14**, superoxide dismutase **1.15.11**, ferrioxidasase, for example ceruloplasmin **1.16.3.1**, especially preferred being the enzymes of class **1.10** which act on related compounds. They catalyze the oxidation of biphenols and ascorbates. Suitable acceptors are  $\text{NAD}^+$ ,  $\text{NADP}^+$  (**1.10.1**), cytochrome (**1.10.2**), oxygen (**1.10.3**) or others (**1.10.99**). Among these, particularly preferred as acceptors are the enzymes of class **1.10.3** with oxygen ( $\text{O}_2$ ) as acceptor.

Particularly preferred among the enzymes of this class are catechol oxidase (tyrosinase) (1.10.3.1), L- ascorbate oxidase (1.10.3.3), O-aminophenol oxidase (1.10.3.4) and laccase (benzenediol: oxygen oxidoreductase) (1.10.3.2), the laccases (benzenediol: oxygen oxidoreductase) (1.10.3.2) being particularly preferred.

Other particularly preferred enzymes are those of group 1.11 which act on a peroxide as acceptor. Only subclass (1.11.1) contains peroxidases. Especially preferred here are cytochrome C peroxidases (1.11.1.5), catalase (1.11.1.6), peroxidase (1.11.1.7), iodide peroxidase, (1.11.1.8), glutathione peroxidase (1.11.1.9), chloride peroxidase (1.11.1.10), L-ascorbate peroxidase (1.11.1.11), phospholipid hydroperoxide glutathione peroxidase (1.11.1.12), manganese peroxidase (1.11.1.13) and diarylpropane peroxidase (ligninase, lignin peroxidase) (1.11.1.14).

b) At least one suitable oxidant,

c) At least one mediator selected from the group consisting of hydroxylamines, hydroxylamine derivatives, hydroxamic acids, hydroxamic acid derivatives, aliphatic, cycloaliphatic, heterocyclic or aromatic compounds containing at least one N-hydroxy, oxime, N-oxo or N,N'-dioxy function and/or at least one mediator from the group of amides, such as, for example, hydrazides or 1,2,4-triazolidin- 3,5-diones (urazoles) and/or at least one mediator from the group of imides such as, for example, the hydantoins, and/or at least one mediator from the group of oxocarbons. Moreover, it is possible to use at least one **mediation enhancer** selected from the group consisting of carbonyl compounds, aliphatic ethers, phenol ethers or olefins (alkenes), and/or at least one **mediation enhancer** selected from the group consisting of the abovesaid mediators of the NO-, NOH- or HRN-OH type and/or amides such as the hydrazides or urazoles and/or the imides such as the hydantoins and/or the oxocarbons.

It is also possible to use at least one mediation enhancer selected from the group consisting of cation radical-generating substances of the phenothiazine and/or phenoxazine type and/or of the (R = N-N = R) type\* (for example, ABTS) or of aryl-substituted alcohols (nonphenols) such as, for example, veratryl alcohol and/or phenol derivatives, such as p-hydroxycinnamic acid, 2,4-dichlorophenol, p-hydroxybenzenesulfonate, vanillin (4-hydroxy-3-methoxybenzaldehyde), p-hydroxybenzoic acid, 5- amino-2-hydroxybenzoic acid (5-aminosalicylic acid) and/or Wurster-type radical cation compounds [see Angewandte Chemie 91, pp. 982-997 (1979); Chem. Unserer Zeit 12, pp. 89-98 (1978); Römpf Chemie Lexikon [Römpf Chemical Encyclopedial, 9th edition, 1995) and/or radical anions, for example semiquinones formed by enzymatic oxidation of hydroquinones.

It is essential for improving the performance of the enzyme/mediator systems with the aid of comediators that the mediator/comediator ratio be from 5000 : 1 to 1 : 1, a ratio from 500 : 1 to 1 : 1 being particularly preferred. When several mediators and comediators are used at the same time, the ratio of these mediator or comediator concentrations depends on the particular combinations employed.

\* N means nitrogen, R denotes groups.

These enzymatic oxidation systems according to the invention contain at least one oxidant. Suitable oxidants are, for example, air, oxygen, ozone, peroxides, such as  $\text{H}_2\text{O}_2$ , organic peroxides, per acids such as peracetic, performic, persulfuric, pernitric, metachloroperoxybenzoic and perchloric acid, per compounds such as perborates, percarbonates or persulfates, or oxygen species and the radicals thereof such as the  $\text{OH}$ ,  $\text{OOH}$  and  $\text{OH}^\cdot$  radical, superoxide ( $\text{O}_2^\cdot$ ), dioxygenyl cation  $\text{O}_2^+$ , singlet oxygen, ozonide ( $\text{O}_3^\cdot$ ), dioxiranes, dioxitanes or Fremy radicals.

Said preferred mediator/mediation enhancer substances of formulas I to XXII and the said other mediation-enhancing compounds are represented in Appendix IV and Appendix IVa.

In the following, by way of an example of enzymatic pulp bleaching, the improvement in performance is presented that can be achieved for many types of pulps by use of a combination of the enzyme component system (ECS) of the invention and the above-described oxidation systems with enzyme action-enhancing compounds.

#### **EXAMPLE 18 (Enzyme: lipase/laccase)**

##### **Enzymatic Bleaching of Softwood (Sulfate Pulp)**

5 g, absolutely dry basis, of wood pulp ( $\text{O}_2$ -delignified softwood), pulp consistency 30% (about 17 g moist) was added to solutions prepared as follows:

A) To 20 mL of tap water were added 1 mg of tetradecanoic acid, 5 mg of benzophenone, 2.5 mg of  $\text{H}_2\text{O}_2$  (30%), 37  $\mu\text{moles}$  of violuric acid + 0.37  $\mu\text{mole}$  of 4-tert.butylurazole with agitation. The pH was adjusted with 0.5 mole/L sulfuric acid solution so that, after addition of the wood pulp and the enzyme, the pH was 5.

B) To 5 mL of tap water was added 5 mg of lipase from *Humicola lanuginosa* (about 25,000 IU), and to this mixture was added an amount of laccase from *Trametes versicolor* sufficient to give an activity of 15 U (1 U = conversion of 1  $\mu\text{mole}$  of ABTS/min/mL of enzyme) per gram of wood pulp. Solutions A and B were combined and diluted to 33 mL. After addition of the wood pulp, the material was mixed in a dough mixer for 2 minutes. The material was then transferred to a reaction vessel preheated to 45 °C and allowed to incubate 1-4 hours at atmospheric pressure.

The pulp was washed over a nylon screen (30 $\mu\text{m}$ ) and extracted for one hour at 60 °C, 2% pulp consistency and using 8% NaOH per gram of wood pulp. The pulp was again washed, after which the kappa number was determined. For results see Table 7.

# **EXAMPLE 19 (Enzyme: lipase/peroxidase)**

## **Enzymatic Bleaching of Softwood (Sulfate Pulp)**

5 g, absolutely dry basis, of wood pulp (O<sub>2</sub>-delignified softwood), pulp consistency 30% (about 17 g moist) was added to solutions prepared as follows:

A) To 20 mL of tap water were added 1 mg of tetradecanoic acid, 5 mg of benzophenone, 2.5 mg of H<sub>2</sub>O<sub>2</sub> (30%), 37 μmoles of violuric acid + 0.37 μmole of 4-tert.butylurazole with agitation. The pH was adjusted with 0.5 mole/L sulfuric acid solution so that, after addition of the wood pulp and the enzyme, the pH was 7.

B) To 5 mL of tap water was added 5 mg of lipase from *Humicola lanuginosa* (about 25,000 IU) and 0.1 mg of peroxidase (horseradish) per gram of wood pulp. Solutions A and B were combined and diluted to 33 mL. After addition of the wood pulp, the material was mixed in a dough mixer for 2 minutes. The material was then transferred to a reaction vessel preheated to 45 °C and allowed to incubate 1-4 hours at atmospheric pressure.

The pulp was washed over a nylon screen (30 μm) and extracted for one hour at 60 °C, 2% pulp consistency and using 8% NaOH per gram of wood pulp. The pulp was again washed, after which the kappa number was determined. For results see Table 7.

**TABLE 7**

<b>SYSTEM</b>	<b>% DELIG. (lipase/lacc.)</b>	<b>% DELIG. (lipase/peroxid.)</b>
ECS + lipase system (+ laccase + violuric acid) +*	44	---
ECS + lipase system (+ peroxidase + violuric acid) +*	---	38
Laccase (+ violuric acid) +*	27	---
Peroxidase (+ violuric acid) +*	---	28

\* **Mediation enhancer**    4-tert.butylurazole

## APPENDIX I

### System Component 2 of the Enzyme Component System (ECS) of the Invention

The fatty acids used in the process of the invention as a source of peracid are, for example:

#### 1) Saturated fatty acids

Butanoic acid	(butyric acid)
Pentanoic acid	(valeric acid)
Hexanoic acid	(caproic acid)
Heptanoic acid	(enanthic acid)
Octanoic acid	(caprylic acid)
Nonanoic acid	(pelargonic acid)
Decanoic acid	(capric acid)
Undecanoic acid	
Dodecanoic acid	(lauric acid)
Tridecanoic acid	
Tetradecanoic acid	(myristic acid)
Pentadecanoic acid	
Hexadecanoic acid	(palmitic acid)
Heptadecanoic acid	
Octadecanoic acid	(stearic acid)
Nonadecanoic acid	
Eicosanoic acid	(arachic acid)
Heneicosanoic acid	
Docosanoic acid	(behenic acid)
Tricosanoic acid	
Tetracosanoic acid	(lignoceric acid)
Pentacosanoic acid	
Hexacosanoic acid	(cerotic acid)
Octacosanoic acid	
Triacosanoic acid	(melissic acid)

#### 2) Unsaturated fatty acids

10-Undecenoic acid	
9-cis-Dodecenoic acid	(lauroleic acid)
9-cis-Tetradecenoic acid	(myristoleic acid)
9-cis-Hexadecenoic acid	(palmistoleic acid)
6-cis-Octadecenoic acid	(petroselinic acid)
6-trans-Octadecenoic acid	(petroselinic acid)
9-cis-Octadecenoic acid	(oleic acid)
9-trans-Octadecenoic acid	(elaidic acid)
9-cis, 12 cis-Octadecadienoic acid	(linoleic acid)
9-trans, 1 2-trans-Octadecadienoic acid	(linolaidic acid)
9-cis, 12-cis, 15-cis-Octadecatrienoic acid	(linolenic acid)
9-trans, 11 -trans, 1 3-trans- Octadecatrienoic acid	( $\alpha$ -eleostearic acid)
9-cis, 11 -trans, 1 3-trans-Octadecatrienoic acid	( $\beta$ -eleostearic acid)

9-cis-Icosenic acid	(gadoleic acid)
Icosa-5,8,11,14-tetraenoic acid	(arachidic acid)
13-cis-Docosenoic acid	(erucic acid)
13-trans-Docosenoic acid	(brassicic acid)
4,8,12,15,19-Docosapentaenoic acid	(clupanodonic acid)

### 3) Polyunsaturated fatty acids

9,12-Octadecadienoic acid	(linoleic acid)
9,12,15-Octadecatrienoic acid	(linolenic acid)
5,9,12-Octadecatrienoic acid	(eleostearic acid)
9,11,13-Octadecatrienoic acid	(parinaric acid)
9,11,13,15-Octadecatetraenoic acid	
5,11,14-Icosatrienoic acid	
5,8,11,14-Icosatetraenoic acid	(arachidic acid)
4,8,12,15,18-Icosapentaenoic acid	(clupanodonic acid)
4,8,12,15,19-Docosapentaenoic acid	(nisinic acid)
4,8,12,15,18,21-Tetracosahexaenoic acid	

Particularly preferred are tetradecanoic acid (myristic acid) and dodecanoic acid (lauric acid).

## APPENDIX II

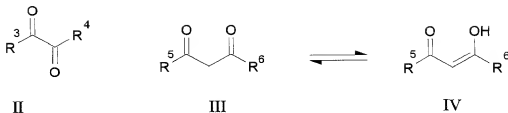
### System Component 4 (Ketones) of the Enzyme Component System (ECS) of the Invention

Particularly preferred are carbonyl compounds of general formula I:



The  $\text{R}^1$  and  $\text{R}^2$  groups can be equal or different and denote aliphatic or aromatic groups. Moreover, the  $\text{R}^1$  and  $\text{R}^2$  groups can form a ring containing besides carbon also heteroatoms such as nitrogen, oxygen and sulfur.

Particularly preferred are 1,2-diketones (formula II), 1,3-diketones (formula III), polyketones (polyketides) and the tautomeric enols (formula IV):



wherein the  $R^3$  to  $R^6$  groups, once again, can be equal or different and denote aliphatic or aromatic groups. Moreover, groups  $R^3$  and  $R^4$  and groups  $R^5$  and  $R^6$ , together, can form a ring containing besides carbon also heteroatoms such as nitrogen, oxygen or sulfur. The possibility of tautomerization or formation of a resonance hybrid is particularly important in this case.

Besides general carbonyl compounds, particularly preferred are ketones, such as, in general hydroxyketones,  $\alpha,\beta$ -unsaturated ketones, oxycarboxylic acids, quinones and halogenated ketones.

Particularly preferred among these are the following:

Acetone, methyl ethyl ketone, diethyl ketone, methyl n-butyl ketone, methyl isobutyl ketone, cyclohexanone, cyclopentanone, 2-methylcyclohexanone, 3-methylcyclohexanone, 4-methylcyclohexanone, dihydroxyacetone, diacetyl monohydrazone, diacetyl dihydrazone, acetophenone, p-hydroxyacetophenone, 1-phenyl-3-butanone, 3-pentanone, 4-heptanone, 2-nonanone, cycloheptanone, cyclooctanone, cyclodecanone, cyclododecanone, dimethyl ketone, ethyl propyl ketone, methyl amyl ketone, acetylacetone, pinacolone, methyl isopropyl ketone, methyl isoamyl ketone, ethyl amyl ketone diisopropyl ketone, diisobutyl ketone, methyl vinyl ketone, methyl isopropenyl ketone, mesityl oxide, isophorone, hydroxyacetone, methoxyacetone, 2,3-pentanedione, 2,3-hexanedione, phenylacetone, propiophenone, benzophenone, benzoin, benzil, 4,4'-dimethoxybenzil, 4'-methoxyacetophenone, 3'-methoxyacetophenone, O-ethylbenzoin, (2-methoxyphenyl)acetone, (4-methoxyphenyl)acetone, methoxy-2-propanone, glyoxylic acid, benzyl glyoxylate, benzylacetone, methyl benzyl ketone, methylcyclohexyl ketone, 2-decanone, dicyclohexyl ketone, 3,3-dimethyl-2-butanone, methyl isobutyl ketone, methyl isopropyl ketone, 2-methyl-3-heptanone, 5-methyl-3-heptanone, 6-methyl-5-hepten-2-one, 5-methyl-2-hexanone, 3-nonanone, 5-nonanone, 2-octanone, 3-octanone, 2-undecanone, 1,3-dichloroacetone, 1-hydroxy-2-butanone, 3-hydroxy-2-butanone, 4-hydroxy-4-methyl-2-pentanone, 2-(1S)-adamantanone, anthrone, bicyclo(3.2.0)hept-2-en-6-one, cis-bicyclo(3.3.0)octan-3,7-dione, (1S)-(-)-camphor, p-chloranil, cyclobutanone, 1,3-cyclohexanedione, 1,4-cyclohexanedione monoethylene ketal, dibenzosuberone, ethyl 4-oxocyclohexanecarboxylate, 9-fluorenone, 1,3-indandione, methylcyclohexanone, phenylcyclohexanone, 4-propylcyclohexanone, 1,2,3,4-tetrahydro-1-naphthalenone, 1,2,3,4-tetrahydro-2-naphthalenone, 3,3,5-trimethylcyclohexanone, 3-acetoxy-2-cyclohexen-1-one, benzylideneacetone, (R)-(-)-carvone,

(S)-(-)carvone, curcumin, 2-cyclohexen-1-one, 2,3-diphenyl-2-cyclopropen-1-one, 2-hydroxy-3-methyl-2-cyclopentene-1-one, isophorone,  $\alpha$ -ionone,  $\beta$ -ionone, 3-methoxy-2-cyclohexen-1-one, 3-methyl-2-cyclopenten-1-one, 3-methyl-3-penten-2-one, (R)-(+)-pulegone, tetraphenyl-2,4-cyclopentadien-1-one, 2,6,6-trimethyl-2-cyclohexen-1,4-dione, 2-acetylbenzoic acid, 1-acetylnaphthalene, 2-acetylnaphthalene, 3'-aminoacetophenone, 4'-aminoacetophenone, 4'-cyclohexylacetophenone, 3',4'-diacetoxyacetophenone, diacetylbenzene, 2',4'-dihydroxyacetophenone, 2',5'-dihydroxyacetophenone, 2',6'-dihydroxyacetophenone, 3,4-dimethoxyacetophenone, 2'-hydroxyacetophenone, 4'-hydroxyacetophenone, 3'-methoxyacetophenone, 4'-methoxyacetophenone, 2'-methylacetophenone, 4'-methylacetophenone, 2'-nitroacetophenone, 3'-nitroacetophenone, 4'-phenylacetophenone, 3,4',5'-trimethoxyacetophenone, 4'-aminopropiophenone, benzoylacetone, benzoylpropionic acid, benzylideneacetophenone, cyclohexyl phenyl ketone, desoxybenzoin, 4',4'-dimethoxybenzil, 1,3-diphenyl-1,3-propanedione, ethylbenzoyl acetate, ethyl phenylglyoxylate, 4'-hydroxypropiofenone, 1,3-indandione, 1-indanone, isopropyl phenyl ketone, 6-methoxy-1,2,3,4-tetrahydronaphthalen-1-one, methylphenyl glyoxylate, phenylglyoxylonitrile, 1-phenyl-1,2-propanedione 2-oxime, valerophenone, 2-acetyl- $\gamma$ -butyrolactone, 2-acetylpyrrole, 1-benzylpiperidin-4-one, dehydroacetic acid, 3,4-dihydro-4,4-dimethyl-2H-pyran-2-one, 1,4-dihydro-4-pyridinone, N-ethoxycarbonyl-4-piperidinone, 2-methyl furyl ketone, 5-hydroxy-2-hydroxymethyl-4H-pyran-4-one, 3-hydroxy-2-methyl-4-pyranone, 3-indolyl methyl ketone, isatin, 1-methyl-4-piperidinone, methyl 2-pyridyl ketone, methyl 3-pyridyl ketone, methyl 4-pyridyl ketone, methyl 2-thienyl ketone, phenyl 2-pyridyl ketone, phenyl 4-pyridyl ketone, tetrahydrofuran-2,4-dione, tetrahydro-4H-pyran-4-one, 2,2,6,6-tetramethyl-4-piperidone, xanthone, acenaphthene quinone, pyruvic acid, (1 R)-(-)-camphor quinone, (1S)-(+)-camphor quinone, 3,5-ditert-butyl-o-benzoquinone, 1,2-dihydroxy-3,4-cyclobutendione, ethyl (2-amino-4-thiazolyl)glyoxylate, ethyl pyruvate, 2,3-hexanedione, 3,4-hexanedione, 3-methyl-2-oxobutyric acid, 3-methyl-2-oxovaleric acid, 4-methyl-2-oxovaleric acid, 2-oxobutyric acid, 2,3-pentandione, 9,10-phenanthrene quinone, acetoacetanilide, 2-acetyl- $\gamma$ -butyrolactone, 2-acetylcyclopentanone, allyl acetoacetate, benzoylacetone, tert-butyl acetoacetate, 1,3-cyclopentanedione, diethyl 3-oxoglutarate, dimethyl acetylsuccinate, dimethyl 3-oxoglutarate, 1,3-diphenyl-1,3-propanedione, ethyl acetoacetate, ethyl benzoylacetate, ethyl butyrylacetate, ethyl 2-oxocyclohexanecarboxylate, ethyl 2-phenylacetoacetate, methyl acetoacetate, 2-methyl-1,3-cyclohexanedione, 2-methyl-1,3-cyclopentanedione, methyl isobutyrylacetate, methyl 3-oxopentanoate, methyl pivaloylacetate, 3-oxoglutaric acid, tetrahydrofuran-2,4-dione, 2,2,6,6-tetramethyl-3,5-heptanedione, 3-benzoylpropionic acid, 1,4-cyclohexanedione, dimethyl acetylsuccinate, ethyl levulinate, 2-aminoanthraquinone, anthraquinone, p-benzoquinone, 1,4-dihydroxyanthraquinone, 1,8-dihydroxyanthraquinone, 2-ethylanthraquinone, methyl-p-benzoquinone, 1,4-naphthoquinone, tetramethyl-p-benzoquinone, 2,2-dimethyl-1,3-dioxan-4,6-dione, 2-benzoylbenzoic acid, 3-benzoylpropionic acid, 5,6-dimethoxyphthalaldehydic acid, levulinic acid, methyl trans-4-oxo-2-pentenoate, phthalaldehydic acid, terephthalaldehydic acid, dibutyl maleate, dibutyl succinate, dibutyl phthalate, dicyclohexyl phthalate, diethyl acetamidomalonate, diethyl adipate, diethyl benzylmalonate, diethyl butylmalonate, diethylethoxymethylenemalonate, diethyl ethylmalonate, diethyl fumarate, diethyl glutarate, diethyl isopropylidenemalonate, diethyl maleate, diethyl malonate, diethyl methylmalonate,



diethyl oxalate, diethyl 3-oxoglutarate, diethyl phenylmatonate, diethyl phthalate, diethyl pimelate, diethyl sebacate, diethyl suberate, diethyl succinate, diisobutyl phthalate, dimethyl acetylene- dicarboxylate, dimethyl acetylsuccinate, dimethyl adipate, dimethyl 2-aminoterephthalate, dimethyl fumarate, dimethyl glutaconate, dimethyl glutarate, dimethyl isophthalate, dimethyl malonate, dimethylmethoxy-malonate, dimethyl methylenesuccinate, dimethyl oxalate, dimethyl 3-oxoglutarate, dimethyl phthalate, dimethyl succinate, dimethyl terephthalate, ethylene glycol diacetate, ethylene glycol dimethacrylate, monoethyl fumarate, monomethyl malonate, monoethyl adipate, monomethyl phthalate, monomethyl pimelate, monomethyl terephthalate, 1,2-propylene glycol diacetate, triethyl methanetricarboxylate, trimethyl 1,2,3-propanetricarboxylate, 3-acetoxy-2-cyclohexen-1-one, allyl acetoacetate, allyl cyanoacetate, benzyl acetoacetate, tert.butyl acetoacetate, butyl cyanoacetate, chlorogenic acid hemihydrate, coumarin-3-carboxylic acid, diethyl ethoxy-carbonylmethanephosphonate, dodecyl gallate, dodecyl 3,4,5-trihydroxybenzoate, (2,3-epoxypropyl) methacrylate, (2-ethoxyethyl) acetate, ethyl acetamidocycanoacetate, ethyl 2-aminobenzoate, ethyl 3-aminopyrazol-4-carboxylate, ethyl benzoxylacetate, ethyl butyrylacetate, ethyl cyanoacetate, ethyl 2-cyano-3-ethoxyacrylate, ethyl cyanofornate, ethyl 2-cyanopropionate, ethyl 3,3-diethoxypropionate, ethyl 1,3-dithian-2-carboxylate, ethyl 2-ethoxyacetate, ethyl 2-furancarboxylate, ethyl levulinate, ethyl mandelate, ethyl gallate, ethyl 2-methylacetate, ethyl 4- nitrocinnamate, ethyl oxamate, ethyl 2-oxocyclohexanecarboxylate, ethyl 4-oxocyclohexane- carboxylate, ethyl 5-oxohexanoate, ethyl 2-phenylacetate, ethyl 4-piperidinedicarboxylate, ethyl 2-pyridinedicarboxylate, ethyl 3-pyridinedicarboxylate, ethyl 4-pyridinedicarboxylate, ethyl thioglycolate, ethyl 3,4,5-trihydroxybenzoate, 2-hydroxyethyl methacrylate, 2-hydroxypropyl methacrylate, 3-indole acetate, 2-methoxyethyl acetate, 1-methoxy-2-propyl acetate, methyl 2- aminobenzoate, methyl 3-aminocrotonate, methyl cyanoacetate, methyl 4-cyanobenzoate, methyl 4- formylbenzoate, methyl 2-furancarboxylate, methyl isobutyrylacetate, methyl methoxyacetate, methyl 2-methoxybenzoate, methyl 3-oxopentanoate, methyl phenylglyoxylate, methyl phenyl-sulfinylacetate, methyl pivatoylacetate, methyl 3-pyridinedicarboxylate, 5-nitrofurfurylidene diacetate, propyl gallate, propyl 3,4,5-trihydroxybenzoate, methyl 3-methylthiopropionate, acetamide, acetanilide, benzamide, benzanilide, N,N-diethylacetamide, N,N-dimethylformamide, N,N-diethyl-3-methyl- benzamide, diethyltoluamide, N,N-dimethylacetamide, N,N-diphenylacetamide, N-methylformamide, N-methylformanilide, N-acetylthiourea, adipic acid diamide, 2-aminobenzamide, 4-aminobenzamide, succinic acid diamide, malonic acid diamide, N,N'-methylene diacrylamide, oxalic acid diamide, pyrazine-2-carboxamide, pyridine-4-carboxamide, N,N,N',N'-tetramethylsuccinic acid diamide, N,N,N',N'-tetramethylglutaric acid diamide, acetoacetanilide, benzohydroxamic acid, cyanoacetamide, 2-ethoxybenzamide, diethyl acetamidomalonate, ethyl acetamidocycanoacetate, ethyl oxamate, hippuric acid Na salt, N-(hydroxy-methyl)acrylamide, L-(-)-lactamide, 2'-nitroacetanilide, 3'- nitroacetanilide, 4'-nitroacetanilide, paracetamol, piperine, salicylanilide, 2-acetyl- $\gamma$ -butyrolactone,  $\gamma$ -butyrolactone,  $\epsilon$ -caprolactone, dihydrocoumarin, 4-hydroxycoumarin, 2-(5H)-furanone, 2,5-dihydro-5-methoxy-2-furanone, phthalide, tetrahydrofuran-2,4-dione, 2,2,6-trimethyl-1,3-dioxin-4-one,  $\gamma$ - valerolactone, 4-amino-1,3-dimethyluracil, barbituric acid, O-benzoyloxycarbonyl-N-hydroxysuccinimide, succinimide, 3,6-dimethyl-piperazin-2,5-dione, 5,5-diphenylhydantoin, ethyl 1,3-dioxoisindoline-2-carboxylate,

9-fluorenylmethylsuccinimidyl carbonate, hydantoin, maleimide, 3-methyl-1-phenyl-2-pyrazolin-5-one, 1-methyl-2-pyrrolidone, methyluracil, 6-methyluracil, oxindole, phenytoin, 1-(2H)-phthalazinone, phthalimide, 2,5-piperazinedione, 2-piperidinone, 2-pyrrolidone, rhodanine, saccharin, 1,2,3,6-tetrahydrophthalimide, 1,2,3,4-tetrahydro-6,7-dimethoxyquinazolin-2,4-dione, 1,5,5-trimethyl-hydantoin, 1-vinyl-2-pyrrolidone, ditert.butyl dicarbonate, diethyl carbonate, dimethyl carbonate, dimethyl dicarbonate, diphenyl carbonate, 4,5-diphenyl-1,3-dioxol-2-one, 4,6-diphenylthieno-(3,4-d)-1,3-dioxo[-2-one 5,5-dioxide, ethylene carbonate, magnesium methoxide methyl carbonate, monomethyl carbonate Na salt, propenyl carbonate, N-allylurea, azodicarbonamide, N-benzylurea, biuret, 1,1'-carbonyldiimidazol, N,N-dimethylurea, N-ethylurea, N-formylurea, urea, N-methylurea, N-phenylurea, 4-phenylsemicarbazide, tetramethylurea, semicarbazide hydrochloride, diethyl azodicarboxylate, methyl carbamate, 1-(4-methoxyphenyl)-2-(2-methoxyphenoxy)ethanone and 1-(4-methoxyphenyl)-2-(2-methoxyphenoxy)ethanol.

Also preferred are anhydrides, such as the following:

Benzoic anhydride, benzene-1,2,4,5-tetracarboxylic acid-1,2,4,5-dianhydride, 3,3',4,4'-benzophenonetetracarboxylic anhydride, succinic anhydride, butyric anhydride, crotonic anhydride, cis-1,2-cyclohexanedicarboxylic anhydride, di-tert-butyl dicarbonate, dimethyl dicarbonate, dodecenylsuccinic anhydride, Epicon B 4400, acetic anhydride, glutaric anhydride, hexanoic anhydride, isatoic anhydride, isobutyric anhydride, isovaleric anhydride, maleic anhydride, 1,8-naphthalenedicarboxylic anhydride, 3-nitrophthalic anhydride, 5-norbornene-2,3-dicarboxylic anhydride, phthalic anhydride, 2-phenylbutyric anhydride, pivalic anhydride, propionic anhydride, cis-1,2,3,6-tetrahydrophthalic anhydride and valeric anhydride.

Particularly preferred are benzophenones such as the following:

Benzophenone, 4-aminobenzophenone, 2-amino-5-chlorobenzophenone, benzophenone-2-carboxylic acid, (S)-(-)-2-(N-benzopropyl)aminobenzophenone, 4,4'-bis(dimethylamino)benzophenone, 4,4'-bis(diethylamino)benzophenone, 3,4-dimethoxybenzophenone, 4,4'-dihydroxybenzophenone, 2,4-dihydroxybenzophenone, 4-hydroxybenzophenone, 2-hydroxy-4-methoxybenzophenone, 4-methoxybenzophenone, 4,4'-dimethoxybenzophenone, 2,2',4,4'-tetrahydroxybenzophenone and 2-chlorobenzophenone.

## APPENDIX III

### Polymerization Catalysts: Phenolic Compounds, Phenol Derivatives or Other Phenolic Polycyclic Compounds with a Number of Oxidizable Hydroxyl Groups

**Preferably, such polymerization catalysts are, for example, the following:**

alizarin, 5-amino-2-hydroxybenzoic acid, 3-aminophenol, pyrocatechol, 2,2-bis(4-hydroxyphenyl)-propane, bis(4-hydroxyphenyl)methane, quinalizarin, 4-chloro-1-naphthol, coniferyl alcohol, 2,4-di-aminophenol dihydrochloride, 3,5-dichloro-4-hydroxyaniline, 1,4-dihydroxyanthraquinone, 2,2-di-hydroxybiphenyl, 4,4-dihydroxybiphenyl, 2,3-dihydroxynaphthalene, 2,6-diisopropylphenol, 3,5-di-methoxy-4-hydroxybenzhydrazine, 2,5-ditert.butylhydroquinone, 2,6-ditert.butyl-4-methylphenol, 4-hydroxybiphenyl, 2-hydroxydiphenylmethane, 2-(2-hydroxyphenyl)benzothiazole, 5-indanol, 2-iso-propoxyphenol, 4-isopropyl-3-methylphenol, 5-isopropyl-2-methylphenol, 4-isopropylphenol, lauryl gallate, 2-naphthol, 4-nonylphenol, 3-(pentadecyl)phenol, 2-propylphenol, 4-propylphenol, purpurine, pyrogallol, 4-(1,1,3,3-tetramethylbutyl)phenol, 1,2,4-trihydroxybenzene, 2,4,6-trimethylphenol, 2,3,5-trimethylphenol, 2,3,6-trimethylphenol, 3,4,5-trimethylphenol, 6,7-dihydroxy-4-methyl coumarin, 2-(2-hydroxyethoxy)benzaldehyde, 1-naphthol, nordihydroguaiaretic acid, octyl gallate, silibinin, 3,4,6-trihydroxybenzoate-octylester, 2,4,6-tritert.butylphenol, 2,4-ditert.butylphenol, 2,6-dichlorophenol, indophenol, ethoxyquin, 1-aminoanthraquinone, 2-amino-5-chlorobenzophenone, 4-aminodiphenylamine, 7-amino-4-hydroxy-2-naphthalenesulfonic acid, 2-(4-aminophenyl)-6-ethylbenzothiazole, benzanthrone, trioctyl trimellitate, trans-chalcone, bis(4-aminophenyl)amine sulfate, 2,2'-ethylidenebis(4,6-ditert.butylphenol), 2,2-bis(2,6-dibromo-4-(2-hydroxyethoxyphenyl)propane, bis(3,5-ditert.butyl-4-hydroxyphenyl)methane, 2,2-bis(3,5-dichloro-4-hydroxyphenyl)propane, Bismarck Brown Y, 1-bromophthalein, 4-butylniline, 2-tert.butyl-5-methylphenol, 1-chloro-anthraquinone, 2-chloro-anthraquinone, triallyl 1,3,5-benzenetricarboxylate, 1,1,1-tris(hydroxy-methyl)propane, tri-methacrylate, pentaerythritol triacrylate, 1,2,4-trivinylcyclohexane, trans,cis-cyclododeca-1,5,9-triene, pentaerythritol tetrabenzoate, 4,4'-methylene-bis(2,6-ditert.butylphenol), 4,4'-isopropylidene-bis(2,6-dichlorophenol), 4,4'-isopropylidene-bis(2,6-dibromophenol), 4,4'-isopropylidene-bis[2-(2,6-dibromophenoxy)ethanol, 2,2'-ethylidene-bis(4,6-ditert.butylphenol), 3-tert.butyl-4-hydroxy-5-methylphenol, 5-tert.butyl-4-hydroxy-2-methylphenol, syringaldazine, 4,4'-dimethoxytriphenylmethane and di-sec.butylphenol.

**Also particularly preferred are compounds with several hydroxyl groups, such as:** ellagic acid, gallic acid, gallein, gallangin, myoinositol, morin, nitrannilic acid, phenolphthalein, purpurin, purpurogallin, quinzarin, chrysazin, quercetin, quinhedrone, chloranilic acid, carmine, rhodizone acid, croconic acid, meilitic acid, hematoxylin, 9-phenyl-2,3,7-trihydroxy-6-fluorene, 9-methyl-2,3,7-trihydroxy-6-fluorene, tetrahydroxy-p-benzoquinone, 2,2',4,4'-tetrahydroxybenzophenone, Pyragallol Red, 1-nitrophloroglucinol, 1,4-dihydroxyanthraquinone, 5,8-dihydroxy-1,4-naphthoquinone, hexa-oxocyclohexane octahydrate, 5,7-dihydroxyflavanone, 3',4'-dihydroxyflavanone,

glyoxal hydrate, 1,3,5-tris(2-hydroxyethyl)isocyanuric acid, quinalizarin and 2,4,5-trihydroxybenzamine.

## APPENDIX IV

Appendix IV shows the formulas of mediators/mediation enhancers (NO<sub>2</sub><sup>-</sup>, NOH- and HNR-OH compounds) which according to the invention can be added to the enzyme component system (ECS) together with oxidoreductases, such as, for example:

**Hydroxylamines (linear or cyclic, aliphatic or aromatic, heterocyclic) of general formula I)**



(I)

**(Amended)** The substituents R<sup>1</sup> and R<sup>2</sup>, which may be the same or different, independently of one other represent one of the following groups: hydrogen, C<sub>1</sub>-C<sub>12</sub> alkyl, carbonyl C<sub>1</sub>-C<sub>6</sub> alkyl, phenyl, aryl, of which C<sub>1</sub>-C<sub>12</sub> alkyl, carbonyl C<sub>1</sub>-C<sub>6</sub> alkyl, phenyl, aryl groups may be unsubstituted or may also be substituted once or multiple times with the radical R<sup>3</sup>.

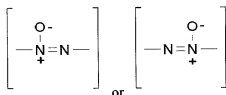
The radical R<sup>3</sup> may represent one of the following groups: hydrogen, halogen, hydroxyl, formyl, carboxy and salts and esters thereof; amino, nitro, C<sub>12</sub>-C<sub>12</sub> alkyl, C<sub>1</sub>-C<sub>6</sub> alkyloxy, carbonyl C<sub>1</sub>-C<sub>6</sub> alkyl, phenyl, sulfono, their esters and salts, sulfamoyl, carbamoyl, phospho, phosphono, phosphonoxy and their salts and esters. The amino, carbamoyl and sulfamoyl groups of the radical R<sup>3</sup> may be unsubstituted or may be substituted once or two times with hydroxy, C<sub>1</sub>-C<sub>3</sub> alkyl, C<sub>1</sub>-C<sub>3</sub> alkoxy. The radicals R<sup>1</sup> and R<sup>2</sup> can jointly form a group -B-. In that case, -B- represents one of the following groups: -(CHR<sup>4</sup>)<sub>n</sub>, (CR<sup>4</sup>=CH-)<sub>m</sub>, n represents an integer from 1 to 6 and m represents an integer from 1 to 3. R<sup>4</sup> is a substituent that is defined like R<sup>3</sup>.

such as compounds of general formula II



(II)

(Amended) X stands for one of the following groups:  $(-\text{N}=\text{N}-)_p$ ,  $(-\text{N}=\text{CR}^{10}-)_p$ ,  $(-\text{CR}^{10}=\text{N}-)_p$ ,  $(-\text{CR}^{11}=\text{CR}^{12}-)_p$ ,



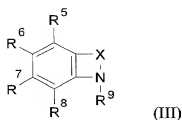
and p is equal to 1 or 2.

The radicals  $\text{R}^9$  to  $\text{R}^{12}$ ,  $\text{R}^{15}$  and  $\text{R}^{16}$  may be the same or different and independently of one another can represent one of the following groups: hydrogen, halogen, hydroxyl, formyl, carboxyl and salts and esters thereof, amino, nitro,  $\text{C}_1$ - $\text{C}_{12}$  alkyl,  $\text{C}_1$ - $\text{C}_6$  alkyloxy, carbonyl  $\text{C}_1$ - $\text{C}_6$  alkyl, phenyl, sulfono, esters and salts thereof, sulfamoyl, carbamoyl, phospho, phosphono, phosphonoxy and their salts and esters. The amino, carbamoyl and sulfamoyl groups of the radicals  $\text{R}^9$  to  $\text{R}^{12}$ ,  $\text{R}^{15}$  and  $\text{R}^{16}$  may be unsubstituted or may also be substituted once or two times with hydroxyl,  $\text{C}_1$ - $\text{C}_3$  alkyl,  $\text{C}_1$ - $\text{C}_3$  alkoxy. The radicals  $\text{R}^{15}$  and  $\text{R}^{16}$  can form a common group -G-, -G- represents one of the following groups:  $(-\text{CR}^5=\text{CR}^6-\text{CR}^7=\text{CR}^8-)$  or  $(-\text{CR}^8=\text{CR}^7-\text{CR}^6=\text{CR}^5-)$ .

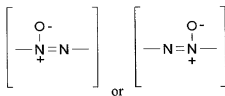
The radicals  $\text{R}^5$  to  $\text{R}^8$  may be the same or different and independently of one another can represent one of the following groups: hydrogen, halogen, hydroxyl, formyl, carboxyl and salts and esters thereof, amino, nitro,  $\text{C}_1$ - $\text{C}_{12}$  alkyl,  $\text{C}_1$ - $\text{C}_6$  alkyloxy, carbonyl,  $\text{C}_1$ - $\text{C}_6$  alkyl, phenyl, sulfono, esters and salts thereof, sulfamoyl, carbamoyl, phospho, phosphono, phosphonoxy and their salts and esters. The amino, carbamoyl and sulfamoyl groups of the radicals  $\text{R}^5$  to  $\text{R}^8$  may be unsubstituted or may also be

substituted once or two times with hydroxy,  $C_1-C_3$  alkyl,  $C_1-C_3$  alkoxy. The  $C_1-C_{12}$  alkyl,  $C_1-C_6$  alkoxy, carbonyl  $C_1-C_6$  alkyl, phenyl and aryl groups of radicals  $R^5$  to  $R^8$  may be unsubstituted or be substituted one or two times with the radical  $R^{18}$ . The radical  $R^{18}$  can represent one of the following groups: hydrogen, halogen, hydroxyl, formyl, carboxyl and their salts and esters, amino, nitro,  $C_1-C_{12}$  alkyl,  $C_1-C_6$  alkoxy, carbonyl  $C_1-C_6$  alkyl, phenyl, aryl, and their esters and salts. The carbamoyl, sulfamoyl and amino groups of the radical  $R^{18}$  may be unsubstituted or may also be substituted once or two times with the radical  $R^{19}$ . The radical  $R^{19}$  may represent one of the following groups: hydrogen; hydroxyl, formyl, carboxyl and their salts and esters; amino, nitro,  $C_1-C_{12}$  alkyl,  $C_1-C_6$  alkoxy, carbonyl  $C_1-C_6$  alkyl, phenyl and aryl.

such as compounds of general formula III:



(Amended) X stands for one of the following groups:  $(-N=N-)$ ,  $(-N=CR^{10}-)_p$ ,  $(-CR^{10}=N-)_p$ ,  $(-CR^{11}=CR^{12}-)_p$

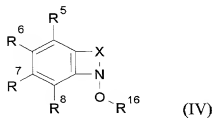


and p is equal to 1 or 2.

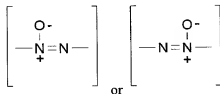
The radicals  $R^5$  to  $R^{12}$  may be the same or different and independently of one another can represent one of the following groups: hydrogen, halogen, hydroxyl, formyl, carboxyl and salts and esters thereof, amino, nitro,  $C_1-C_{12}$  alkyl,  $C_1-C_6$  alkoxy, carbonyl  $C_1-C_6$  alkyl, phenyl, aryl, sulfono esters and salts thereof, sulfamoyl,

carbamoyl, phospho, phosphono, phosphonoxy and their salts and esters. The amino, carbamoyl and sulfamoyl groups of the radicals  $R^5$  to  $R^{12}$  may be unsubstituted or may also be substituted once or two times with hydroxyl,  $C_1$ - $C_3$  alkyl,  $C_1$ - $C_3$  alkoxy. The  $C_1$ - $C_{12}$  alkyl,  $C_1$ - $C_6$  alkoxy, carbonyl  $C_1$ - $C_6$  alkyl, phenyl, aryl, aryl  $C_1$ - $C_6$  alkyl groups of radicals  $R^5$  to  $R^{12}$  may be unsubstituted or substituted once or two times with the radical  $R^{13}$ . The radical  $R^{13}$  can represent one of the following groups: hydrogen, halogen, hydroxyl, formyl, carboxyl and their salts and esters; amino, nitro,  $C_1$ - $C_{12}$  alkyl,  $C_1$ - $C_6$  alkoxy, carbonyl  $C_1$ - $C_6$  alkyl, phenyl, aryl, sulfono, sulfeno, sulfinio, and their esters and salts. The carbamoyl, sulfamoyl, amino groups of the radical  $R^{13}$  may be unsubstituted or may also be substituted once or two times with the radical  $R^{14}$ . The radical  $R^{14}$  may represent one of the following groups: hydrogen; hydroxyl, formyl, carboxyl and their salts and esters; amino, nitro,  $C_1$ - $C_{12}$  alkyl,  $C_1$ - $C_6$  alkoxy, carbonyl  $C_1$ - $C_6$  alkyl, phenyl or aryl.

such as compounds of general formula IV:



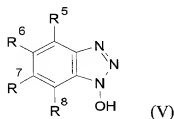
(Amended) X stands for one of the following groups:  $(-N=N-)$ ,  $(-N=CR^{10}-)_m$ ,  $(-CR^{10}=N-)_m$ ,  $(-CR^{11}=CR^{12}-)_m$



and m is equal to 1 or 2.

For the radicals  $R^5$  to  $R^8$  and  $R^{10}$  to  $R^{12}$  what has been said above applies.  $R^{17}$  can be hydrogen,  $C_1$ - $C_{10}$  alkyl,  $C_1$ - $C_{10}$  carbonyl, of which  $C_1$ - $C_{10}$  alkyl and  $C_1$ - $C_{10}$  carbonyl can be unsubstituted or mono- or polysubstituted with a radical  $R^{18}$ , which is defined like  $R^3$ .

**such as compounds, namely derivatives, of 1-hydroxybenzotriazole and the tautomeric benzotriazole 1-oxide, as well as the esters and salts thereof, particularly compounds of formula V :**



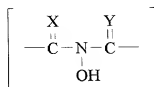
**(Amended)** The radicals  $R^5$  to  $R^8$  may be the same or different and independently of one another can represent one of the following groups: hydrogen, halogen, hydroxyl, formyl, carboxyl and salts and esters thereof; amino, nitro,  $C_1$ - $C_{12}$  alkyl,  $C_1$ - $C_6$  alkyloxy, carbonyl  $C_1$ - $C_6$  alkyl, phenyl, sulfono esters and salts thereof, sulfamoyl, carbamoyl, phospho, phosphono, phosphonooxy and their salts and esters. The amino, carbamoyl and sulfamoyl groups of the radicals  $R^5$  to  $R^8$  may be unsubstituted or may also be substituted once or two times with hydroxyl,  $C_1$ - $C_3$  alkyl,  $C_1$ - $C_3$  alkoxy. The  $C_1$ - $C_{12}$  alkyl,  $C_1$ - $C_6$  alkyloxy, carbonyl  $C_1$ - $C_6$  alkyl, phenyl or aryl groups of radicals  $R^5$  to  $R^8$  may be unsubstituted or may also be substituted one or mono- or polysubstituted with the radical  $R^{18}$ . The radical  $R^{18}$  can represent one of the following groups: hydrogen, halogen, hydroxyl, formyl, carboxyl and their salts and esters; amino, nitro,  $C_1$ - $C_{12}$  alkyl,  $C_1$ - $C_6$  alkyloxy, carbonyl  $C_1$ - $C_6$  alkyl, phenyl, aryl, sulfono, sulfeno, sulfinio, and their esters and salts. The carbamoyl, sulfamoyl, amino groups of the radical  $R^{18}$  may be unsubstituted or may also be substituted once or two times with the radical  $R^{19}$ . The radical  $R^{19}$  may represent one of the following groups: hydrogen; hydroxyl, formyl, carboxyl and their salts and esters, amino, nitro,  $C_1$ - $C_{12}$  alkyl,  $C_1$ - $C_6$  alkyloxy, carbonyl  $C_1$ - $C_6$  alkyl, phenyl or aryl.



such as, for example, the following compounds:

1 -hydroxybenzotriazole  
 1 -hydroxybenzotriazole, sodium salt  
 1 -hydroxybenzotriazole, potassium salt  
 1 -hydroxybenzotriazole, lithium salt  
 1 -hydroxybenzotriazole, ammonium salt  
 1 -hydroxybenzotriazole, calcium salt  
 1 -hydroxybenzotriazole, magnesium salt  
 1 -hydroxybenzotriazole-6-sulfonic acid, monosodium salt  
 1-methoxy-1 H-benzotriazole  
 1 -acetoxy- 1 H-benzotriazole  
 1 -hydroxy-(4,5-f)-dioxolo-1 H-benzotriazole  
 1 -hydroxy-6-methyl-3H-benzotriazole  
 1-hydroxy-6-nitro-1 H-benzotriazole  
 1 -hydroxy-5,6-dimethyl-1 H-benzotriazole  
 1 -hydroxy-6-methoxy-1 H-benzotriazole  
 1 -hydroxy-5,6-dimethoxy-1 H-benzotriazole  
 1 -hydroxy-1 H-benzotriazole-6-carboxylic acid  
 1,5-dihydroxy-1 H-benzotriazole  
 1 -hydroxy-1 H-benzotriazole-6-sulfonic acid hydrazide  
 1 -hydroxy-1 H-benzotriazole-6-carboxamide  
 1 -hydroxy-5-methoxy-1 H-benzotriazole  
 6-amino-1 -hydroxy-1 H-benzotriazole  
 6-amino-5-methoxy-1 H-benzotriazole  
 6-chloro- 1 -hydroxy- 1 H-benzotriazole  
 6-acetamido-1 -hydroxy-1 H-benzotriazole  
 1 -hydroxy- 1 H-benzotriazole-6-carboxylic acid ethyl ester  
 1 -hydroxy-4-nitro-1 H-benzotriazole  
 4-chloro-1 -hydroxy-1 H-benzotriazole  
 1 -hydroxy-6-tert. butyl- 1 H-benzotriazole  
 6-cyclohexyl-1 -hydroxy-1 H-benzotriazole  
 6-isopropyl-1 -hydroxy-1 H-benzotriazole  
 1 -hydroxy-6-phenyl-1 H-benzotriazole  
 3-methyl-3H-benzotriazole 1 -oxide  
 2-phenyl-2H-benzotriazole 1 -oxide

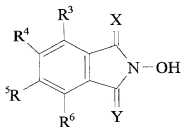
such as compounds of general formula A (cyclic N-hydroxy compounds):



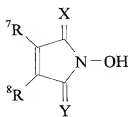
(A)

**(Amended)** as well as their salts, ethers or ester, in which X and Y are the same or different and stand for O, S or NR<sup>1</sup>, in which R<sup>1</sup> stands for hydrogen, hydroxyl, formyl, carbamoyl, or sulfonyl radical, or ester or salt of the sulfonyl radical, sulfamoyl, nitro, amino, phenyl, aryl C<sub>1</sub>-C<sub>5</sub> alkyl, C<sub>1</sub>-C<sub>12</sub> alkyl, C<sub>1</sub>-C<sub>5</sub> alkoxy, C<sub>1</sub>-C<sub>10</sub> carbonyl, carbonyl C<sub>1</sub>-C<sub>6</sub> alkyl, phospho, phosphono or phosphonooxy radical, or ester or salt of the phosphonooxy radical, and carbamoyl, sulfamoyl, amino and phenyl radicals may be unsubstituted or substituted once or multiple times with a radical R<sup>2</sup>, and the aryl C<sub>1</sub>-C<sub>5</sub> alkyl, C<sub>1</sub>-C<sub>12</sub> alkyl, C<sub>1</sub>-C<sub>5</sub> alkoxy, C<sub>1</sub>-C<sub>10</sub> carbonyl, carbonyl C<sub>1</sub>-C<sub>6</sub> alkyl radicals may be saturated or unsaturated, branched or unbranched, and substituted once or multiple times with a radical R<sup>2</sup> and R<sup>2</sup> is the same or different and stands for hydroxyl, formyl, or carboxyl radical, ester or salt of the carboxyl radical, carbamoyl, sulfonyl ester or salt of the sulfonyl radical, sulfamoyl, nitro, amino, phenyl, C<sub>1</sub>-C<sub>5</sub> alkyl, C<sub>1</sub>-C<sub>5</sub> alkoxy radical.

Such as compounds of general formula VI, VII, VIII or IX:

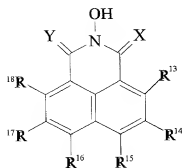
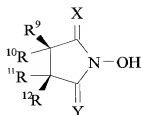


VI



VII

VIII

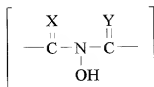


IX

**(Amended)** in which X, Y have the meanings already given and the radicals  $R^3$  to  $R^{18}$  are the same or different and stand for halogen radical, carboxy radical, salt or ester of a carboxy radical, or the meaning given for  $R^1$ ,

in which  $R^9$  and  $R^{10}$ , or  $R^{11}$  and  $R^{12}$ , must not at the same time stand for a hydroxyl or amino radical and optionally two at a time of the substituents  $R^3$  to  $R^6$ ,  $R^7$  to  $R^8$ ,  $R^9$  to  $R^{12}$ ,  $R^{13}$  to  $R^{18}$  can be linked together into a ring -B-, in which -B- has one of the following meanings:

$(-CH=CH)_n$ , where  $n = 1$  to  $3$ ,  $-CH=CH-CH=N-$ , or



(A)

**(Amended)** and in which optionally the radicals  $R^9$  to  $R^{12}$  may also be linked to one another by one or two bridge elements -Q-, in which -Q- may be the same or different and can have the following meanings: -O-, -S-,  $\text{CH}_2$ -,  $-\text{CR}^{19}=\text{CR}^{20}$ -, in which  $R^{19}$  and  $R^{20}$  are the same or different and have the same meaning as  $R^3$ , and in which X and Y stand for O or S.

**for example, compounds such as:**

N-hydroxyphthalimide and optionally substituted N-hydroxyphthalimide derivatives, N-hydroxymaleimide and optionally substituted N-hydroxymaleimide derivatives, N-hydroxynaphthalimide and optionally substituted N-hydroxynaphthalimide derivatives, N-hydroxysuccinimide and optionally substituted N-hydroxysuccinimide derivatives, **such as, for example:**

N-hydroxyphthalimide, N-hydroxybenzene-1,2,4-tricarboximide, N,N'-dihydroxypyromellitic acid diimide, N,N'-dihydroxybenzophenone-3,3',4,4'-tetracarboxylic acid diimide, **for example, (formula VII):**

N-hydroxymaleimide, pyridine-2,3-dicarboxylic acid N-hydroximide

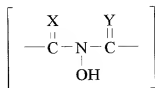
**for example (formula VIII):**

N-1-hydroxysuccinimide, N-1-hydroxytartarimide, N-hydroxy-5-norbornene-2,3-dicarboximide, exo-N-hydroxy-7-oxabicyclo[2.2.1]-5-heptene-2,3-dicarboximide, N-hydroxy-cis-cyclohexane-1,2-dicarboximide, N-hydroxy-cis-4-cyclohexene-1,2-dicarboximide

**for example (formula IX):**

N-hydroxynaphthalimide sodium salt

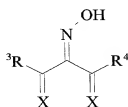
**for example (six-membered ring of formula A):**



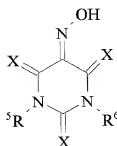
(A)

N-hydroxyglutarimide

such as compounds of general formula X or XI (oximes):



X



XI

(Amended) and their salts, ethers or esters, in which X is the same or different and stands for O, S or NR<sup>1</sup>, in which R<sup>1</sup> stands for hydrogen, hydroxyl, formyl, carbamoyl, or sulfono radical, or ester or salt of the sulfono radical, sulfamoyl, nitro, amino, phenyl, acryl C<sub>1</sub>-C<sub>5</sub> alkyl, C<sub>1</sub>-C<sub>12</sub> alkyl, C<sub>1</sub>-C<sub>5</sub> alkoxy, C<sub>1</sub>-C<sub>10</sub> carbonyl, carbonyl C<sub>1</sub>-C<sub>6</sub> alkyl, phospho, phosphono or phosphonoxy radical, or ester or salt of the phosphonoxy radical, in which carbamoyl, sulfamoyl, amino and phenyl radicals may be unsubstituted or substituted once or multiple times with a radical R<sup>2</sup>, and the aryl C<sub>1</sub>-C<sub>5</sub> alkyl, C<sub>1</sub>-C<sub>12</sub> alkyl, C<sub>1</sub>-C<sub>5</sub> alkoxy, C<sub>1</sub>-C<sub>10</sub> carbonyl, carbonyl C<sub>1</sub>-C<sub>6</sub> alkyl radicals may be saturated or unsaturated, branched or unbranched, and substituted once or multiple times with a radical R<sup>2</sup>, and R<sup>2</sup> is the same or different and stands for hydroxy, formyl, or carboxy radical, ester or salt of the carboxy radical, carbamoyl, sulfono ester or salt of the sulfono radical, sulfamoyl, nitro, amino, phenyl, C<sub>1</sub>-C<sub>5</sub> alkyl, C<sub>1</sub>-C<sub>5</sub> alkoxy radical, and the radicals R<sup>3</sup> and R<sup>4</sup> are the same or different and stand for halogen, carboxyl radical, ester or salt of the carboxyl radical, or have the meanings given for R<sup>1</sup>, or are linked together into a ring (-CR<sup>7</sup>R<sup>8</sup>)<sub>n</sub>, where n is equal to 2, 3 or 4, and R<sup>5</sup> and R<sup>6</sup> have the

meanings given for R<sup>1</sup>, and R<sup>7</sup> and R<sup>8</sup> are the same or different and stand for halogen, carboxyl radical, ester or salt of the carboxyl radical, or have the meanings given for R<sup>1</sup> and in which in formula X, X can stand for O or S and the other radicals have the meanings named above.

**for example (formula X):**

dimethyl 2-hydroxyiminomalonate

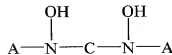
**for example (formula XI):**

1-methylvioluric acid, 1,3-dimethylvioluric acid, thiovioluric acid, alloxane 4,5-dioxime, alloxane 5- oxime hydrate (violuric acid) and/or the esters or salts thereof,

**( Amended) such as compounds from the class of N-aryl-N-hydroxyamides of general formula XII, XIII and XIV: ( XIVa, XIVb, XIVc, XIVd and XIYe:)**



**XII**



**XIII**



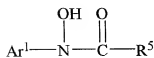
**XIV**

**(Amended) and the salts, ethers and esters thereof, wherein**

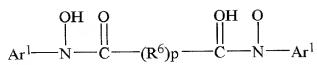
A is a monovalent homoaromatic or heteroaromatic monocyclic or bicyclic group and D is a divalent homoaromatic or heteroaromatic monocyclic or bicyclic group, these aromatics possibly being substituted with one or more equal or different R<sup>1</sup> groups selected from the group consisting of halogen or a hydroxyl, formyl, cyano, carbamoyl

or carboxyl group, an ester or salt of the carboxyl group, a sulfo group, an ester or salt of the sulfo group, or sulfamoyl, nitro, nitroso, amino, phenyl, aryl-C<sub>1</sub>-C<sub>5</sub> alkyl, C<sub>1</sub>-C<sub>12</sub> alkyl, C<sub>1</sub>-C<sub>5</sub> alkoxy, C<sub>1</sub>-C<sub>10</sub> carbonyl, carbonyl-C<sub>1</sub>-C<sub>6</sub> alkyl, phospho, phosphono or phosphonoxy group, or an ester or salt of the phosphonoxy group, and the carbamoyl, sulfamoyl, amino and phenyl groups are unsubstituted or substituted with one or several R<sup>2</sup> groups, and the aryl-C<sub>1</sub>-C<sub>5</sub> alkyl, C<sub>1</sub>-C<sub>12</sub> alkyl, C<sub>1</sub>-C<sub>5</sub> alkoxy, C<sub>1</sub>-C<sub>10</sub> carbonyl, carbonyl-C<sub>1</sub>-C<sub>6</sub> alkyl groups are saturated or unsaturated, branched or unbranched, and can be substituted with one or more R<sup>2</sup> groups, with the R<sub>2</sub> groups being equal or different and denoting a hydroxyl, formyl, cyano or carboxyl group, an ester or a salt of the carboxyl group, or a carbamoyl, sulfonyl, sulfamoyl, nitro, nitroso, amino, phenyl, C<sub>1</sub>-C<sub>5</sub> alkyl, C<sub>1</sub>-C<sub>5</sub> alkoxy or C<sub>1</sub>-C<sub>5</sub> alkylcarbonyl group and pairs of R<sup>1</sup> or R<sup>2</sup> groups are joined by a (-CR<sup>3</sup>R<sup>4</sup>)<sub>m</sub> link, with m equal to 1, 2, 3 or 4 and R<sup>3</sup> and R<sup>4</sup> are equal or different and denote a carboxyl group, an ester or salt of a carboxyl group, a phenyl, C<sub>1</sub>-C<sub>5</sub> alkyl, C<sub>1</sub>-C<sub>5</sub> alkoxy or C<sub>1</sub>-C<sub>5</sub> alkylcarbonyl group and one or more of the nonadjacent (-CR<sup>3</sup>R<sup>4</sup>-) groups can be replaced with oxygen, sulfur or an optionally C<sub>1</sub>-C<sub>5</sub> alkyl group-substituted imino group, and two adjacent (-CR<sup>3</sup>R<sup>4</sup>-) groups can be replaced by a (-CR<sup>3</sup>=R<sup>4</sup>-) group, and B denotes a monovalent acid radical, in the amide form, of an acid selected from the group consisting of carboxylic acids with up to 20 carbon atoms, carbonic acid, half-esters of carbonic or carbamic acid, sulfonic acid, phosphonic acid, phosphoric acid, monoesters of phosphoric acid or diesters of phosphoric acid. And C denotes a divalent acid radical, in the amide form, of an acid selected from the group consisting of monocarboxylic and dicarboxylic acids with up to 20 carbon atoms, carbonic acids, sulfonic acids, phosphonic acids, phosphoric acids and monoesters of phosphoric acid.

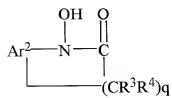
(Amended) such as compounds from the class of N-aryl-N-hydroxyamides of general formula (XII, XIII and XIV:) XIVa, XIVb, XIVc, XIVd and XIVE:



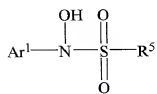
XIVa



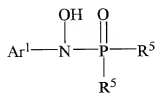
**XIVb**



**XIVc**



**XIVd**



**XIVe**



**(Amendends)** and the salts, ethers and esters thereof, wherein  $Ar^1$  denotes a homoaromatic or heteroaromatic monocyclic aryl group and  $Ar^2$  is a divalent homoaromatic or heteroaromatic monocyclic aryl group, possibly substituted with one or more equal or different  $R^7$  groups selected from among hydroxy, cyano or carboxyl groups, or esters or salts of the carboxyl group, or the sulfo group or esters or salts of the sulfo group, or the nitro, nitroso, amino,  $C_1$ - $C_{12}$  alkyl,  $C_1$ - $C_5$  alkoxy,  $C_1$ - $C_{10}$  carbonyl, carbonyl- $C_1$ - $C_6$  alkyl group, wherein the amino groups are unsubstituted or substituted with one or more  $R^8$  groups and the  $C_1$ - $C_{12}$  alkyl,  $C_1$ - $C_5$  alkoxy,  $C_1$ - $C_{10}$  carbonyl, carbonyl- $C_1$ - $C_6$  alkyl groups are saturated or unsaturated, branched or unbranched and possibly are substituted with one or more  $R^8$  groups, said groups being equal or different and denoting a hydroxyl or carboxyl group, an ester or salt of the carboxyl group, a sulfo, nitro, amino,  $C_1$ - $C_5$  alkyl,  $C_1$ - $C_5$  alkoxy or  $C_1$ - $C$  alkylcarbonyl group, and pairs of  $R^7$  groups are connected by a  $(-CR^3R^4)_m$  link, with m equal to 0, 1, 2, 3 or 4, and  $R^3$  and  $R^4$  have the aforesaid meaning, and one or more of the adjacent  $(-CR^3R^4-)$  groups are possibly substituted with oxygen, sulfur or an imino group optionally substituted with a  $C_1$ - $C_5$  alkyl group, and two adjacent  $(-CR^3R^4-)$  groups are substituted with a  $(-CR^3=R^4-)$  group, and  $R^3$  denotes equal or different monovalent groups selected from among phenyl, aryl- $C_1$ - $C_5$  alkyl,  $C_1$ - $C_{12}$  alkyl,  $C_1$ - $C_5$  alkoxy and  $C_1$ - $C_{10}$  carbonyl groups, the phenyl groups possibly being unsubstituted or substituted with one or more  $R^9$  groups, and the aryl- $C_1$ - $C_5$  alkyl,  $C_1$ - $C_{12}$  alkyl,  $C_1$ - $C_5$  alkoxy and  $C_1$ - $C_{10}$  carbonyl groups are saturated or unsaturated, branched or unbranched and possibly substituted with one or more  $R^9$  groups, wherein the  $R^9$  groups are equal or different and denote a hydroxyl, formyl, cyano or carboxyl group or an ester or salt of the carboxyl group, or a carbamoyl, sulfo, sulfamoyl, nitro, nitroso, amino, phenyl,  $C_1$ - $C_5$  alkyl or  $C_1$ - $C_{12}$  alkoxy group, and  $R^6$  is a divalent group selected from among the ortho, meta and para-phenylene, aryl- $C_1$ - $C_5$  alkyl,  $C_1$ - $C_{12}$  alkylene and  $C_1$ - $C_5$  alkylenedioxy groups, the phenylene groups possibly being unsubstituted or substituted with one or more  $R^9$  groups, and the aryl- $C_1$ - $C_5$  alkyl,  $C_1$ - $C_{12}$  alkyl and  $C_1$ - $C_5$  alkoxy groups possibly being saturated or unsaturated, branched or unbranched and possibly substituted with one or more  $R^9$  groups, and wherein p stands for 0 or 1 and q stands for an integer of 1 to 3.

Preferably,  $Ar^1$  denotes a phenyl group and  $Ar^2$  an ortho-phenylene group,  $Ar^1$  possibly being substituted with up to five and  $Ar^2$  with up to four equal or different groups selected from among  $C_1$ - $C_3$  alkyl or  $C_1$ - $C_3$  alkylcarbonyl groups or carboxyl groups or sulfo groups, or esters or salts of the sulfo group, or a hydroxyl, cyano, nitro, nitroso or amino group, wherein the amino groups are possibly substituted with two different groups selected from among the hydroxyl and  $C_1$ - $C_3$  alkylcarbonyl groups.

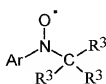
$R^5$  preferably denotes a monovalent group selected from among hydrogen and the phenyl,  $C_1$ - $C_{12}$  alkyl and  $C_1$ - $C_3$  alkoxy groups, wherein the  $C_1$ - $C_{12}$  alkyl groups and the

C<sub>1</sub>-C<sub>3</sub> alkoxy groups are saturated or unsaturated, branched or unbranched.  
R<sup>6</sup> preferably denotes divalent groups selected from among the ortho- or para-phenylene, C<sub>1</sub>-C<sub>12</sub> alkylene, C<sub>1</sub>-C<sub>5</sub> alkylendioxy groups, wherein the aryl-C<sub>1</sub>-C<sub>5</sub> alkyl, C<sub>1</sub>-C<sub>12</sub> alkyl and C<sub>1</sub>-C<sub>5</sub> alkoxy groups are saturated or unsaturated, branched or unbranched and are possibly substituted with one or more R<sup>5</sup> groups.  
Preferably, R<sup>9</sup> denotes a carboxyl group, an ester or salt of the carboxyl group or a carbamoyl, phenyl or C<sub>1</sub>-C<sub>3</sub> alkoxy group.

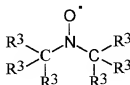
such as compounds of general formula XV, XVI and XVII (nitroxyl radicals/nitroxides):



XV



XVI

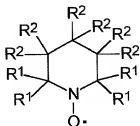


XVII

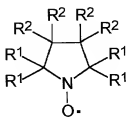
**(Amended)** wherein Ar denotes a monovalent homoaromatic or heteroaromatic monocyclic or bicyclic group and wherein these aromatics are possibly substituted with one or more equal or different R<sub>1</sub> groups selected from among the groups consisting of halogen, formyl, cyano, carbamoyl and carboxyl groups, esters and salts of the carboxyl group, or the sulfo group or esters or salts of the sulfo group, or sulfamoyl, nitro, nitroso, amino, phenyl, aryl-C<sub>1</sub>-C<sub>5</sub> alkyl, C<sub>1</sub>-C<sub>12</sub> alkyl, C<sub>1</sub>-C<sub>5</sub> alkoxy, C<sub>1</sub>-C<sub>10</sub> carbonyl, carbonyl-C<sub>1</sub>-C<sub>6</sub> alkyl, phospho, phosphono or phosphonoxo group or an ester or salt of the phosphonoxo group, and wherein the phenyl, carbamoyl and sulfamoyl groups can be unsubstituted or substituted with one or more R<sup>2</sup> groups, the amino group possibly

being substituted with one or two  $R^2$  groups, and the aryl- $C_1$ - $C_5$  alkyl,  $C_1$ - $C_{12}$  alkyl,  $C_1$ - $C_5$  alkoxy,  $C_1$ - $C_{10}$  carbonyl and carbonyl- $C_1$ - $C_6$  alkyl groups being saturated or unsaturated, branched or unbranched and possibly substituted with one or more  $R^2$  groups, wherein the  $R^2$  groups are present once or several times and are equal or different and denote a hydroxyl, formyl or cyano group or carboxyl group or an ester or salt of the carboxyl group, or a carbamoyl, sulfo, sulfamoyl, nitro, nitroso, amino, phenyl,  $C_1$ - $C_5$  alkyl,  $C_1$ - $C_5$  alkoxy or  $C_1$ - $C_5$  alkylcarbonyl group, and wherein the  $R^3$  groups are present once or several times and are equal or different and denote a halogen or a hydroxyl, mercapto, formyl, cyano, carbamoyl or carboxyl group, or an ester or salt of the carboxyl group, or a sulfo group, or an ester or salt of the sulfo group, or a sulfamoyl, nitro, nitroso, amino, phenyl, aryl- $C_1$ - $C_5$  alkyl,  $C_1$ - $C_{12}$  alkyl,  $C_1$ - $C_5$  alkoxy,  $C_1$ - $C_{10}$  carbonyl, carbonyl- $C_1$ - $C_6$  alkyl, phospho, phosphono or phosphonoxy group or an ester or salt of the phosphonoxy group and wherein  $R^3$  in the case of stable bicyclic nitroxy radicals (structure N) stands also for hydrogen and wherein the carbamoyl, sulfamoyl, amino, mercapto and phenyl groups are unsubstituted or substituted with one or more  $R^4$  groups and the aryl- $C_1$ - $C_5$  alkyl,  $C_1$ - $C_{12}$  alkyl,  $C_1$ - $C_5$  alkoxy,  $C_1$ - $C_{10}$  carbonyl and carbonyl- $C_1$ - $C_6$  alkyl groups are saturated or unsaturated, branched or unbranched and are possibly substituted with one or more  $R^4$  groups, wherein the  $R^4$  groups are equal or different and denote a hydroxyl, formyl, cyano or carboxyl group or an ester or salt of the carboxyl group, or a carbamoyl, sulfo, sulfamoyl, nitro, nitroso, amino, phenyl,  $C_1$ - $C_5$  alkyl,  $C_1$ - $C_5$  alkoxy or  $C_1$ - $C_5$  alkylcarbonyl group, and pairs of  $R^3$  or  $R^4$  groups can be joined by a  $(-R^5R^6)_m$  link with m equal to 0, 1, 2, 3 or 4, and  $R^5$  and  $R^6$  are equal or different and denote halogen, a carboxyl group, an ester or salt of the carboxyl group, or a carbamoyl, sulfamoyl, phenyl, benzoyl,  $C_1$ - $C_5$  alkyl,  $C_1$ - $C_5$  alkoxy or  $C_1$ - $C_5$  alkylcarbonyl group, and one or more nonadjacent  $(-R^5R^6-)$  groups can be replaced with oxygen, sulfur or an optionally  $C_1$ - $C_5$ -alkyl-substituted imino group, and two adjacent  $(-R^5R^6-)$  groups can be replaced with a  $(-R^5=R^6-)$ ,  $(-CR^5=N-)$  or  $(CR^5=N(O)-)$  group,

such as compounds of general formula XVII a and XVII b (nitroxyl radicals):



XVIIa



XVIIb

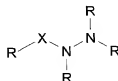
(Amended) wherein the  $R^1$  groups are equal or different and denote phenyl, aryl- $C_1$ - $C_5$ -alkyl,  $C_1$ - $C_{12}$  alkyl,  $C_1$ - $C_5$  alkoxy,  $C_1$ - $C_{10}$  carbonyl or carbonyl- $C_1$ - $C_6$  alkyl groups and the phenyl groups are unsubstituted or substituted with one or more  $R^3$  groups, and the aryl- $C_1$ - $C_5$  alkyl,  $C_1$ - $C_{12}$  alkyl,  $C_1$ - $C_5$  alkoxy,  $C_1$ - $C_{10}$  carbonyl or carbonyl- $C_1$ - $C_6$  alkyl groups are saturated or unsaturated, branched or unbranched and are possibly substituted with one or more  $R^3$  groups, wherein the  $R^3$  groups can be present once or several times, and are equal or different denoting a hydroxyl, formyl or carboxyl group or an ester or salt of the carboxyl group, or a carbamoyl, sulfo, sulfamoyl, vitro, nitroso, amino, phenyl, benzoyl,  $C_1$ - $C_5$  alkyl,  $C_1$ - $C_5$  alkoxy or  $C_1$ - $C_5$  alkylcarbonyl group, and the  $R^2$  groups are present once or several times and are equal or different, denoting hydrogen or a hydroxyl, mercapto, formyl, cyano, carbamoyl or carboxyl group or an ester or salt of the carboxyl group, or a sulfo group or an ester or salt of the sulfo group, or a sulfamoyl, nitro, nitroso, amino, phenyl, aryl- $C_1$ - $C_5$  alkyl,  $C_1$ - $C_{12}$  alkyl,  $C_1$ - $C_5$  alkoxy,  $C_1$ - $C_{10}$  carbonyl, carbonyl- $C_1$ - $C_6$  alkyl, phospho, phosphono or phosphonoxo group or an ester or salt of the phosphonoxo group, and wherein the carbamoyl, sulfamoyl, amino, mercapto and phenyl groups are unsubstituted or substituted with one or more  $R^3$  groups, and the aryl- $C_1$ - $C_5$  alkyl,  $C_1$ - $C_{12}$  alkyl,  $C_1$ - $C_5$  alkoxy,  $C_1$ - $C_{10}$  carbonyl or carbonyl- $C_1$ - $C_6$  alkyl groups are saturated or unsaturated, branched or unbranched and possibly are substituted with one or more  $R^3$  groups, and one  $(-CR^2R^2-)$  group can be replaced with oxygen, an optionally  $C_1$ - $C_5$  alkyl-substituted imino group, a hydroximino group, a carbonyl function or an optionally mono or disubstituted vinylidene function, and two adjacent  $(-CR^2R^2-)$  groups can be

replaced with a  $(-CR^2=R^2-)$ ,  $(-CR^2=N-)$  or  $(-CR^2=N(O)-)$  group.

such as compounds of general formula XVIIIa (amides) and XVIII b (hydrazides):



XVIIIa



XVIII b

(Amended) wherein X denotes C=O or O=S=O (carboxamides or sulfonamides). The R groups can be equal or different and independently of each can stand for hydrogen, alkyl, aryl or acyl groups (carboxylic acid or sulfonic acid groups).

such as compounds of general formula XVIII c (cyclic hydrazides):



XVIII c

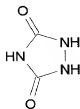
(Amended) wherein X stands for C=O or O=S=O (cyclic hydrazides of dicarboxylic acids or disulfonic acids).

The R groups can be equal or different and independently of each other represent hydrogen, alkyl, aryl or acyl groups.

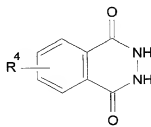
G denotes the following atoms or atomic groups:  $\text{CH}_2$ ,  $\text{CH}_2\text{-CH}_2$ ,  $\text{CHR}^1\text{-CHR}^1$ ,  $\text{CH=CH}$ ,  $\text{CR}^2\text{-CR}^2$ ,  $\text{NH}$ ,  $\text{NR}^3$ , C=O, ortho- $\text{C}_6\text{H}_4$  (ortho-substituted phenyl group), ortho- $\text{C}_{10}\text{H}_6$  (ortho-substituted naphthyl group), wherein the  $\text{R}^1$  to  $\text{R}^3$  groups can be equal or

different and independently of each other denote hydrogen, alkyl, aryl, or acyl groups.

such as urazoles (formula XVIII d) and phthalhydrazides (formula XVIII e):



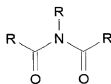
XVIII d



XVIII e

(Amended) wherein R<sup>4</sup> denotes hydrogen, alkyl, alkoxy, carboxyl, nitro or amino groups. The R groups are equal or different and independently of each other stand for hydrogen, alkyl or aryl.

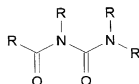
such as compounds of general formula XIX (imides):



XIX

(Amended) The R groups are equal or different and independently of each other denote hydrogen, alkyl, aryl, acyl or amino groups.

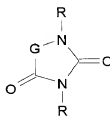
such as compounds of general formula XIXa (imides):



**XIX a**

**(Amended)** The R groups are equal or different and independently of each denote hydrogen, alkyl, aryl, acyl or amino groups.

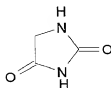
such as compounds of general formula XIXb (cyclic imides):



**XIX b**

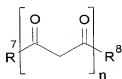
**(Amended)** The R groups are equal or different and independently of each other denote hydrogen, alkyl, aryl, acyl or amino groups, group G representing one of the following atoms or atomic groups:  $\text{CH}_2$ ,  $\text{CHR}^1$ ,  $\text{CHR}^1\text{R}^2$ ,  $\text{CH}=\text{CH}$ ,  $\text{CR}^3-\text{CR}^4$ ,  $\text{NH}$ ,  $\text{NR}^5$ ,  $\text{C}=\text{O}$  or  $\text{O}$ , with  $\text{R}^1$  to  $\text{R}^5$  being equal or different and independently of each other denoting hydrogen, alkyl, aryl, alkoxy or carboxyl groups.

such as compounds of general formula XIXc (hydantoin derivatives):



XIX c

such as compounds of general formula XX, such as  $\alpha$ -hydroxycarbonyl compounds of general formula XXa,  $\alpha$ -dicarbonyl compounds of general formula XXb,  $\beta$ -hydroxycarbonyl compounds of general formula XXc and  $\beta$ -dicarbonyl compounds of general formula XXd:



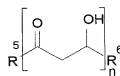
XXa



XX b



XX c

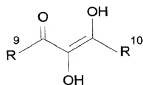


XX d

(Amended) wherein the  $R^1$  to  $R^8$  groups independently of each other denote one of the following atoms or atomic groups: hydrogen, halogen, alkyl, alkyloxy, aryl, aryloxy, hydroxy, oxo, formyl, thioxo, mercapto, alkylthio, sulfeno, sulfino, sulfo, sulfamoyl, amino, imino, amido, amidino, hydroxycarbamoyl, hydroximino, nitroso, nitro or hydrazono group, with  $R^1$  and  $R^2$ ;  $R^3$  and  $R^4$ ;  $R^5$  and  $R^6$ ; and  $R^7$  and  $R^8$  possibly combining to form a single group, and wherein  $n$  is greater than or equal to 1.



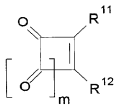
such as compounds of general formula XXI (linear compounds with double bonds/enols):



XXI

(Amended) wherein  $R^9$  and  $R^{10}$  independently of each other denote one of the following atoms or atomic groups: hydrogen, halogen, alkyl, alkyloxy, aryl, aryloxy, hydroxy, oxo, formyl, thioxo, mercapto, alkylthio, sulfeno, sulfino, sulfo, sulfamoyl, amino, imino, amido, amidino, hydroxycarbamoyl, hydroximino, nitroso, nitro or hydrazono, with  $R^9$  and  $R^{10}$  possibly combining to form a single group.

such as compounds of general structure XXII (cyclic compounds, groups not OH, derivatives of squaric acid, OH group derivatized):



XXII

(Amended) wherein the  $R^{11}$  and  $R^{12}$  group independently of each other denote one of the following atoms or atomic groups: hydrogen, halogen, alkyl, alkyloxy, aryl, aryloxy, hydroxy, oxo, formyl, thioxo, mercapto, alkylthio, sulfeno, sulfino, sulfo, sulfamoyl, amino, imino, amido, amidino, hydroxycarbamoyl, hydroximino, nitroso, nitro or hydrazono, and  $m$  is greater than or equal to 0. Particularly preferred are cyclic oxocarbons of the general empirical formula:  $H_2C_xO_x$  and their anions having general formula  $C_xO_x^{2-}$ , wherein  $x$  is greater than or equal to 3.

such as, for example:

deltic acid, squaric acid, croconic acid and rhodizonic acid

(Amended) **\*The formula descriptions (groups/R ... are also given in the German Published, Non Examined Patent Application DE 197 19 857.0. and in the US Patent Application Serial No. 09/029,401**

#### APPENDIX IVa

**Appendix IVa** shows compounds which can be added to the enzyme component system (ECS) of the invention as mediation enhancers, primarily as additives together with mediators and oxidoreductases:

**Aliphatic ethers and aryl-substituted alcohols, such as:**

2,3-dimethoxybenzyl alcohol, 3,4-dimethoxybenzyl alcohol, 2,4-dimethoxybenzyl alcohol, 2,6-dimethoxybenzyl alcohol, homovanillic alcohol, ethylene glycol monophenyl ether, 2-hydroxybenzyl alcohol, 4-hydroxybenzyl alcohol, 4-hydroxy-3-methoxybenzyl alcohol, 2-methoxybenzyl alcohol, 2,5-dimethoxybenzyl alcohol, 3,4-dimethoxybenzylamine, 2,4-dimethoxybenzylamine hydrochloride, veratryl alcohol, coniferyl alcohol.

**olefins (alkenes), for example:**

2-allylphenol, 2-allyl-6-methylphenol, allylbenzene, 3,4-dimethoxypropenylbenzene, p-methoxystyrene, 1-allylimidazole, 1-vinylimidazole, styrene, stilbene, allyl phenyl ether, benzyl cinnamate, methyl cinnamate, 2,4,6-triallyloxy-1,3,5-triazine, 1,2,4-trivinylcyclohexane, 4-allyl-1,2-dimethoxybenzene, 4-tert. benzoic acid vinyl ester, squalene, benzoin allyl ether, cyclohexene, dihydropyran and N-benzylcinnamamide.

**Phenol ethers, such as:**

2,3-dimethoxybenzyl alcohol, 3,4-dimethoxybenzyl alcohol, 2,4-dimethoxybenzyl alcohol, 2,6-dimethoxybenzyl alcohol, homovanillic alcohol, 4-hydroxybenzyl alcohol, 4-hydroxy-3-methoxybenzyl alcohol, 2-methoxybenzyl alcohol, 2,5-dimethoxybenzyl alcohol, 3,4-dimethoxybenzylamine, 2,4-dimethoxybenzylamine hydrochloride, veratryl alcohol, coniferyl alcohol, veratrol, anisole.

**Carbonyl compounds, such as:**

4-aminobenzophenone, 4-acetylphenylbenzophenone, benzil, benzophenone hydrazone, 3,4-dimethoxybenzaldehyde, 3,4-dimethoxybenzoic acid, 3,4-dimethoxybenzophenone, 4-dimethylaminobenzaldehyde, 4-acetylphenyl hydrazone,

benzophenone 4-carboxylic acid, benzoylacetone, bis(4,4'-dimethylamino)benzophenone, benzoin, benzoin oxime, N-benzoyl-N-phenylhydroxylamine, 2-amino-5-chlorobenzophenone, 3-hydroxy-4-methoxybenzaldehyde, 4-methoxybenzaldehyde, anthraquinone-2-sulfonic acid, 4-methylaminobenzaldehyde, benzaldehyde, benzophenone-2-carboxylic acid, 3,3',4,4'-benzophenonetetracarboxylic dianhydride, (S)-(-)-2-(N-benzylpropyl)-aminobenzophenone, benzylphenylacetanilide, N-benzylbenzanilide, 4,4'-bis(dimethylamino)thiobenzophenone, 4,4'-bis(diacetylamino)benzophenone, 2-chlorobenzophenone, 4,4'-dihydroxybenzophenone, 2,4-dihydroxybenzophenone, 3,5-dimethoxy-4-hydroxybenzaldehyde hydrazine, hydroxybenzophenone, 2-hydroxy-4-methoxybenzophenone, 4-methoxybenzophenone, 3,4-dihydroxybenzophenone, p-

anisic acid, p-anisaldehyde, 3,4-dihydroxybenzaldehyde, 3,4-dihydroxybenzoic acid, 2,5-dimethoxy-4-hydroxybenzaldehyde, 3,5-dimethoxy-4-hydroxybenzoic acid, 4-hydroxybenzaldehyde, salicylaldehyde, vanillin, vanillic acid.

## APPENDIX 5

### Possible Oxidation Reactions of the Enzyme Component System

#### 1) Hydroxylation reactions

- Synthesis of alcohols
- Hydroxylation of steroids
- Hydroxylation of terpenes
- Hydroxylation of benzenes
- Hydroxylation of alkanes
- Hydroxylation of aromatic compounds
- Hydroxylation of double bonds
- Hydroxylation of nonactivated methyl groups
- Dihydroxylation of aromatic compounds

#### 2) Oxidation of unsaturated aliphatics

- Preparation of epoxides
- Preparation of compounds by epoxidation
- Preparation of arene oxides
- Preparation of phenols
- Preparation of cis-dihydrodiols

#### 3) Baeyer-Villiger oxidations

- Baeyer-Villiger conversion of steroids

**4) Oxidation of heterocycles**

- a) Transformation of organic sulfides
- b) Oxidation of sulfur compounds
- c) Oxidation of nitrogen compounds (formation of N-oxides etc.)
- d) Oxidation of other heteroatoms

**5) Carbon-carbon dehydrogenation**

- a) Dehydrogenation of steroids

**6) Other oxidation reactions**

- a) Oxidation of alcohols and aldehydes
- b) Oxidation of aromatic methyl groups to aldehydes
- c) Oxidative coupling of phenols
- d) Oxidative degradation of alkyl chains ( $\beta$ -oxidation etc.)
- e) Formation of peroxides or percompounds
- f) Initiation of free-radical induced chain reactions.

CLAIMS

1. Enzyme component system (ECS) as an oxidation and bleaching system for the preparation of special highly selective oxidants, consisting of

- a) system component 1): at least one hydrolase from enzyme classes 3.1, 3.1.1, 3.1.2, 3.1.3, 3.1.4, or 3.1.7 and/or at least one hydrolase from enzyme classes 3.5, 3.5.1, 3.5.2, 3.5.3, 3.5.4, 3.5.5 or 3.5.99,
- b) system component 2): at least one fatty acid, preferably  $C_6$  to  $C_{26}$  (saturated, monounsaturated or polyunsaturated),
- c) system component 3): at least one oxidant precursor for reaction with the enzymes,
- d) system component 4): at least one ketone from the group of carbonyl compounds.

2. Enzyme component system according to Claim 1, characterized in that enzymes of class 3.1.1.3: lipases (triacylglycerol lipase, triglyceroacyl hydrolases) are used as system component 1).

3. Enzyme component system according to Claims 1 and 2, characterized in that enzymes of class 3.5.1.4, amidases, and/or class 3.5.5.1, nitrilases, are used as system component 1).

4. Enzyme component system according to Claims 1 and 2, characterized in that the enzymes of class 3.1.1.3 (lipases) are obtained from organisms such as *Candida antarctica*, *Candida rugosa*, *Candida lipolytica*, *Candida cylindracea*, *Candida spec.*, *Geotrichum candidum*, *Humicola lanuginosa*, *Penicillium cambertii*, *Penicillium roquefortii*, *Aspergillus spec.*, *Mucor javanicus*, *Mucor mehei*, *Rhizopus arrhizus*, *Rhizopus niveus*, *Rhizopus delamar*, *Rhizopus spec.*, *Chromobacterium viscosum*, *Pseudomonas cepacia* and *Pseudomonas spec.* from wheat seedlings or pancreas (pig or other sources).

5. Enzyme component system according to Claims 1 and 3, characterized in that the enzymes of classes 3.5.1.4 and 3.5.5.1 are obtained from organisms such as *Pseudomonas aeruginosa*, *Pseudomonas putida*, *Pseudomonas acidovorax*, *Pseudomonas spec.*, *Aspergillus nidulans*, *Aspergillus spec.*, *Brevibacterium spec.*, *Streptococcus pneumoniae*, *Rhodococcus spec.* and others.

6. Enzyme component system according to Claims 1 through 5, characterized in that it contains enzymes from fungi, bacteria, animals or plants obtained from natural organisms or organisms modified by genetic engineering.

7. Enzyme component system according to Claims 1 through 6, characterized in that modified enzymes, enzyme constituents, prosthetic groups or mimicking substances are used as catalysts.

8. Enzyme component system according to Claims 1 through 7, characterized in that it contains as system component 2) one or more saturated, monounsaturated or polyunsaturated, preferably  $C_6$  to  $C_{26}$  fatty acids according to Appendix 1.

9. Enzyme component system according to Claim 8, characterized in that it contains as system component 2) preferably tetradecanoic acid (myristic acid) and/or dodecanoic acid (lauric acid).

10. Enzyme component system according to Claims 1 through 9, characterized in that it contains as system component 3) at least one oxidant precursor such as peroxide ( $H_2O_2$ ), an organic peroxide such as 3-chloroperoxybenzoic acid, Mg monoperoxyphthalate, di-tert.butyl peroxide, cumene hydroperoxide, lauroyl peroxide, chloroperoxybenzoic acid, dicumyl hydroperoxide, methyl ethyl ketone peroxide, benzoyl peroxide, diperoxydodecanedioic acid Na salt and others and a per compound such as a perborate, persulfate, percarbonate, perphosphate, percarbamide, perchlorate etc.

11. Enzyme component system according to Claims 1 and 10, characterized in that it contains as system component 3) the  $H_2O_2$ -activating ions  $Mo^{6+}$ ,  $W^{6+}$ ,  $Va^{5+}$  and/or compounds such as the nitrilamines and/or dicyandiamides.

12. Enzyme component system according to Claims 1, 10 and 11, characterized in that it preferably contains  $H_2O_2$  as system component 3).

13. Enzyme component system according to Claims 1, 10 and 11, characterized in that it contains as system component 3)  $H_2O_2$  generated in situ from glucose and GOD.

14. Enzyme component system according to Claims 10 through 13, characterized in that it contains as system component 3) besides per acids also a bleaching activator such as TAED (tetraacetythylenediamine), TAGU (tetraacetylglucuril) and iso-NOBS (sodium p-isononanoxyloxybenzenesulfonate).

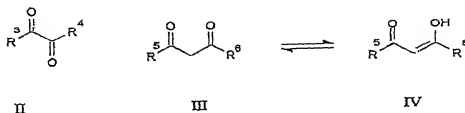
15. Enzyme component system according to Claims 1 and 10 through 14, characterized in that it contains as system component 3) besides the peroxides and/or per compounds also air or oxygen at atmospheric pressure at a slightly positive pressure of up to 2 bar.

16. Enzyme component system according to Claims 1 through 15, characterized in that it contains as system component 4) at least one ketone of general formula I:



wherein the  $R^1$  and  $R^2$  groups are equal or different and denote aliphatic or aromatic groups and furthermore can form a ring containing besides carbon also heteroatoms, such as nitrogen, oxygen and sulfur.

17. Enzyme component system according to Claims 1 through 16, characterized in that it contains as system component 4) a 1,2-diketone of formula II, a 1,3-diketone of formula III or a polyketone (polyketide) as well as a tautomeric enol of formula IV



wherein groups  $R^3$  to  $R^6$ , once again, are equal or different and denote aliphatic or aromatic groups and furthermore groups  $R^3$  and  $R^4$  and groups  $R^5$  and  $R^6$ , together, can form a ring containing besides carbon also heteroatoms such as nitrogen, oxygen or sulfur.

18. Enzyme component system according to Claims 1 through 17, characterized in that it contains as system component 4) besides a general carbonyl compound also a ketone such as, in general, a hydroxyketone,  $\alpha,\beta$ -unsaturated ketone, oxydicarboxylic acid, quinone and halogenated ketone.

19. Enzyme component system according to Claims 1 through 18, characterized in that it contains as system component 4) a compound such as those listed in Appendix 2.

20. Enzyme component system according to Claims 1 through 19, characterized in that it contains a polymerization catalyst such as, in particular, a phenolic substance or polycyclic compound with several oxidizable hydroxyl groups according to Appendix 3.

21. Enzyme component system according to Claims 1 through 20, characterized in that it is possible to add to it as an additional system an enzymatic oxidation system with enzyme action-enhancing compounds, said system containing

- a) at least one suitable oxidation catalyst
- b) at least one suitable oxidant
- c) at least one mediator selected from the group consisting of hydroxylamines, hydroxylamine derivatives, hydroxamic acids, hydroxamic acid derivatives, aliphatic, cycloaliphatic, heterocyclic or aromatic compounds containing at least one N-hydroxy, oxime, N-oxy or N,N'-dioxy function and/or at least one mediator from the group of amides, such as, for example, hydrazides or 1,2,4-triazolidin-3,5-diones (urazoles) and/or at least one mediator from the group of imides such as, for example, the hydantoin, and/or at least one mediator from the group of oxocarbons.

22. Enzyme component system according to Claims 1 through 21, characterized in that it is possible to add to it as an additional system an enzymatic oxidation system with enzyme action-enhancing compounds, said system containing:

at least one mediation enhancer selected from the group consisting of carbonyl compounds, aliphatic ethers, phenol ethers or olefins (alkenes) and/or at least one mediation enhancer selected from the group consisting of NO-, NOH- and HRN-OH compounds and/or amides such as hydrazides or urazoles and/or imides such as hydantoin and/or oxocarbons.

23. Enzyme component system according to Claims 1 through 22, characterized in that it is possible to add to it as an additional system an enzymatic oxidation system with enzyme action-enhancing compounds, said system containing:

at least one mediation enhancer selected from the group consisting of cation radical-generating substances, of the phenothiazine and/or phenoxazine type and/or of the (R=N-N=R) type<sup>10</sup> (for example, ABTS) or of aryl-substituted alcohols (nonphenols) such as, for example, veratryl alcohol and/or phenol derivatives, such as p-hydroxycinnamic acid, 2,4-dichlorophenol, p-hydroxybenzenesulfonate, vanillin (4-hydroxy-3-methoxybenzaldehyde), p-hydroxybenzoic acid, 5-amino-2-hydroxybenzoic acid (5-aminosalicylic acid) and/or Wurster-type radical cation compounds, such as p-phenylenediamine, preferably N,N-dimethyl-p-phenylenediamine, N,N-diethyl-p-phenylenediamine, N,N,N',N'-tetramethyl-p-phenylenediamine, 2,3,5,6-tetramethyl-p-phenylenediamine and/or radical anions, for example semiquinones, which can be generated by enzymatic oxidation of hydroquinones.

24. Enzyme component system according to Claims 1 through 21, characterized in that it is possible to use as oxidation catalysts enzymes such as the oxidoreductases of classes 1.1.1. to 1.97, and preferably:

cellobiose: oxygen-1-oxidoreductase (cellobiose oxidase) (1.1.3.25),  
cellobiose: quinone-1-oxidoreductase (1.1.5.1), bilirubin oxidase (1.3.3.5),  
cytochrome oxidase (1.9.3), oxygenases, lipxygenases (1.13, 1.14),  
superoxide dismutase (1.15.11), ferrioxidase, for example ceruloplasmin (1.16.3.1),  
1.10 such as catechol oxidase (tyrosinase)(1.10.3.1), L-ascorbate oxidase (1.10.3.3), O-aminophenol oxidase (1.10.3.4) and laccase (benzodiol:oxygen oxidoreductase)(1.10.3.2)  
1.11 such as cytochrome C peroxidase (1.11.1.5), catalase (1.11.1.6), peroxidase (1.11.1.7), iodide peroxidase (1.11.1.8), glutathione peroxidase (1.11.1.9), chloride peroxidase (1.11.1.10) and L-ascorbate peroxidase (1.11.1.11), phospholipid hydroperoxide glutathione peroxidase (1.11.1.12), manganese peroxidase (1.11.1.13) and diarylpropane peroxidase (ligninase, lignin peroxidase)(1.11.1.14).

25. Enzyme component system according to Claims 1, 21 and 24, characterized in that enzymes such as laccases and/or peroxidases are preferably used as oxidation catalysts.

26. Enzyme component system according to Claim 25, characterized in that it preferably contains laccases and/or peroxidases from white rotting fungi such as, for example, *Trametes versicolor*, *Trametes spec.*, *Phlebia spec.*, *Pleurotus spec.*, *Phanerochaete chrysosporium*, *Agaricus spec.* etc and also other fungi, bacteria, plant and animal cells obtained from natural organisms or organisms modified by genetic engineering.

27. Enzyme component system according to Claims 1, 21 to 26, characterized in that modified enzymes, enzyme constituents, prosthetic groups or mimicking substances are used as the enzyme catalysts.

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<sup>10</sup> N means nitrogen, R denotes groups.



28. Enzyme component system according to Claims 21 through 27, characterized in that it employs as additional oxidants preferably air, oxygen, ozone, a peroxide such as  $H_2O_2$ , an organic peroxide, a per acid such as peracetic, performic, persulfuric, pernitric, metachloroperoxybenzoic and perchloric acid, a per compound such as a perborate, percarbonate and persulfate, or oxygen species and the radicals thereof such as the OH, OOH and  $OH^*$  radical, superoxide ( $O_2^*$ ), dioxygenyl cation ( $O_2^{+*}$ ), singlet oxygen, ozonide ( $O_3$ ), dioxiranes, dioxitanes or Fremy radicals.

29. Enzyme component system according to Claims 21 through 28, characterized in that the mediators and additional mediation enhancers are those shown in Appendix IV and IVa.

30. Enzyme component system according to Claims 21 through 29, characterized in that the mediator/mediation enhancer ratio is from 5000:1 to 5:1 and preferably from 500:1 to 5:1.

31. Use of the enzyme component system according to Claims 1 to 30 in a process for the delignification and/or modification and/or bleaching of cellulose from wood or annual plants and of wood pulps and deinked pulps, whereby the reaction of the enzyme component system is carried out at a pH from 2 to 11, preferably at pH 3 to 9, at a temperature from 20 to 95 °C, preferably from 40 to 95 °C, at a pulp consistency from 0.5 to 40%, preferably from 4 to 15%, in the presence of oxygen or air at atmospheric pressure or a slightly positive pressure (up to 2 bar), and system component 1, namely lipase from *Hemicula lanuginosa*, is used at a concentration from 0.05 to 5 mg, preferably from 0.05 to 2 mg, and amidase from *Pseudomonas aeruginosa* is used at a concentration from 40 to 200 IU,

and system component 2, namely one or more fatty acids, preferably  $C_8$  to  $C_{18}$  fatty acids, preferably tetradecanoic and/or dodecanoic acid, are used at a concentration from 0.05 to 20 mg, preferably from 0.05 to 10 mg,

and system component 3, namely the oxidant precursor, preferably  $H_2O_2$ , is used at a concentration from 0.05 to 20 mg (100%), preferably from 0.05 to 10 mg,

and system component 4, namely a ketone, preferably benzophenone, is used at a concentration from 0.05 to 20 mg, preferably at a concentration from 0.05 to 10 mg, in each case based on 1 g of absolutely dry pulp.

32. Use of the enzyme component system according to Claims 1 and 31 in a process for the delignification and/or modification and/or bleaching of cellulose from wood or annual plants and of wood pulps and deinked pulps, whereby an acid wash or a Q-step is used before and/or after the reaction of the enzyme component system and the acid wash is carried out at 60-120 °C, at pH 2 to 5.5, for 30-90 min and at 4-20% pulp consistency, and the Q-step is carried out with 0.05-1%, preferably with 0.2 to 0.5% of chelator at 60-100 °C, at pH 2 to 5.5 for 30-90 min and at a pulp consistency of 4-20%.

33. Use of the enzyme component system according to Claims 1, 31 and 32 in a process for the delignification and/or modification and/or bleaching of cellulose from wood or annual plants and of wood pulps and deinked pulps, whereby the acid wash and the Q-step are carried out for 1 hour at 60-90 °C, at pH 2 to 5 and at 10% pulp consistency.

34. Use of the enzyme component system according to Claims 1, 31 to 33 in a process for the delignification and/or modification and/or bleaching of cellulose from wood or annual plants and of wood pulps and deinked pulps, whereby said system can be used before or after any possible treatment of the pulp by single or multiple digestion, bleaching steps or other pre- and post-treatments, such as alkaline leaching, alkaline extraction, washing, acid treatment, O<sub>2</sub>-step, O<sub>2</sub> delignification step, peroxide bleaching step, O<sub>2</sub>-promoted peroxide step, pressure peroxide step, per acid step, per acid-promoted O<sub>2</sub> or peroxide step, ozone bleaching step, dioxirane step, polymethoxalate step, Cl-delignification step, ClO<sub>2</sub> bleaching step, Cl/ClO<sub>2</sub> bleaching step, reductive bleaching steps, sulfonation steps, NO/NO<sub>2</sub> treatments, nitrosylsulfuric acid treatment, swelling steps, enzyme treatments, for example treatments with hydrolases such as cellulases and/or hemicellulases (for example, xylanase, mannanase, etc) and/or pectinases and/or proteinases and/or lipases and/or amidases and/or oxidoreductases, such as, for example, laccases and/or peroxidases etc or several combined treatments.

35. Use of the enzyme component system according to Claims 1 and 34 in a process for the delignification and/or modification and/or bleaching of cellulose from wood or annual plants and wood pulps and deinked pulps, whereby the swelling step is carried out with the aid of substances such as, for example, glycols, such as propylene glycol or ethylene glycol, glycol ethers such as ethylene glycol dimethyl ether etc, but also with the aid of solvents, for example, alcohols such as methanol, ethanol, butanol, amyl alcohol, cyclohexanol, benzyl alcohol and chlorohydrin, phenols such as phenol, methylphenols and methoxyphenols, aldehydes such as formaldehyde and chloral, mercaptans such as butyl mercaptan, benzyl mercaptan and thioglycolic acid, organic acids such as formic, acetic and chloroacetic acid, amines such as ammonia and hydrazine, hydrotropic solvents, for example a concentrated solution of sodium benzoate, other substances such as benzenes, pyridines, dioxane, ethyl acetate, and other basic solvents such as OH<sup>-</sup>/H<sub>2</sub> or OH<sup>-</sup>/alcohol etc.

36. Use of the enzyme component system according to Claims 1 to 35 in a process for the delignification and/or modification and/or bleaching of cellulose from wood or annual plants and of wood pulps and deinked pulps, whereby there is added to the reaction solution a complexing agent such as ethylenediaminetetraacetic acid (EDTA), diethylenetriaminepentaacetic acid (DTPA), hydroxyethylenediaminetriacetic acid (HEDTA), diethylenetriaminopentamethylenephosphonic acid (DTMPA), nitrilotriacetic acid (NTA), polyphosphoric acid (PPA) or other complexing agents for iron, manganese or copper, for example diethylamine or hydroxylamine.

37. Use of the enzyme component system according to Claims 1 to 36 in a process for the delignification and/or modification and/or bleaching of cellulose from wood or annual plants and of wood pulps and deinked pulps, said process being carried out in several steps and whereby between each step is applied a washing or washing and extraction step with alkaline hydroxide solution, or neither washing nor extraction takes place.

38. Use of the enzyme component system according to Claims 1 to 37 in the treatment of paper production wastewater (grinder wastewater, TMP wastewater) and of wastewater from other branches of the industry, such as wood pulp wastewater and textile production wastewater, among others, whereby the reaction of the enzyme component system is carried out at pH 2 to 11, preferably 3 to

6, at a temperature from 20 to 95 °C, preferably from 40 to 95 °C, in the presence of oxygen or air at atmospheric pressure or slightly positive O<sub>2</sub> pressure (up to 2 bar), and system component 1, namely lipase from *Aspergillus spec.* is used at a concentration from 0.05 to 50 mg, preferably from 0.5 to 10 mg, and system component 2, namely one or more fatty acids, preferably C<sub>8</sub> to C<sub>16</sub> fatty acids, preferably tetradecanoic acid and/or dodecanoic acid, is used at a concentration from 0.05 to 200 mg, preferably at a concentration from 0.05 to 50 mg, and system component 3, namely the oxidant precursor, preferably H<sub>2</sub>O<sub>2</sub>, is used at a concentration from 0.05 to 200 mg (100%), preferably at a concentration from 0.05 to 50 mg, and system component 4, namely a ketone, preferably a benzophenone, is used at a concentration from 0.05 to 200 mg, preferably at a concentration from 0.05 to 50 mg, and that a polymerization catalyst, preferably purpurogallin, is used at a concentration from 0.005 to 200 mg, preferably at a concentration from 0.005 to 50 mg, the concentrations in all cases being based on 1 liter of wastewater.

39. Use of the enzyme component system according to Claims 1 to 38 for the production of lignin solutions or gels and of the corresponding binders/adhesives, and for the production of wood-based composites, whereby the reaction of the enzyme component system is carried out at pH 2 to 11, preferably 3 to 6, at a temperature from 20 to 95 °C, preferably from 40 to 95 °C, in the presence of oxygen or air at atmospheric pressure or slightly positive O<sub>2</sub> pressure (up to 2 bar), and system component 1, namely lipase from *Humicola lanuginosa* is used at a concentration from 0.05 to 50 mg, preferably from 0.5 to 10 mg, and system component 2, namely one or more fatty acids, preferably C<sub>8</sub> to C<sub>16</sub> fatty acids, preferably tetradecanoic acid and/or dodecanoic acid, is used at a concentration from 0.05 to 200 mg, preferably at a concentration from 0.05 to 50 mg, and system component 3, namely the oxidant precursor, preferably H<sub>2</sub>O<sub>2</sub>, is used at a concentration from 0.05 to 200 mg (100%), preferably at a concentration from 0.05 to 50 mg, and system component 4, namely a ketone, preferably a benzophenone, is used at a concentration from 0.05 to 200 mg, preferably at a concentration from 0.05 to 50 mg, and that a polymerization catalyst, preferably purpurogallin, is used at a concentration from 0.005 to 200 mg, preferably at a concentration from 0.005 to 50 mg, the concentrations in all cases being based on 1 liter of wastewater.

40. Use of the enzyme component system according to Claims 1 to 39 in a process for the enzymatic printing ink removal during the deinking of waste paper, whereby the reaction of the enzyme component system is carried out at pH 7 to 11, preferably at pH 7 to 9, at a temperature from 20 to 95 °C, preferably from 40 to 95 °C, in the presence of oxygen or air at atmospheric pressure or slightly positive O<sub>2</sub> pressure (up to 2 bar), and system component 1, namely lipase from *Humicola lanuginosa*, is used at a concentration from 5 to 500 mg, preferably from 5 to 100 mg, and system component 2, namely one or more fatty acids, preferably C<sub>8</sub> to C<sub>16</sub> fatty acids, preferably tetradecanoic acid and/or dodecanoic acid, is used at a concentration from 5 to 2000 mg, preferably at a concentration from 5 to 500 mg, and system component 3, namely the oxidant precursor, preferably H<sub>2</sub>O<sub>2</sub>, is used at a concentration from 5 to 5000 mg (100%), preferably at a concentration from 5 to 1000 mg, and system component 4, namely a ketone, preferably a benzophenone, is used at a concentration from 5 to 2000 mg, preferably at a concentration from 5 to 500 mg, and that, to change the optimum pH for the printing ink removal reaction and to affect the swelling behavior of the waste paper, a phenolic or polycyclic substance with several oxidizable hydroxyl groups, preferably bisphenol

A, is used at a concentration from 1 to 2000 mg and from 1 to 500 mg, in each case based on 1 kg of air-dried waste paper.

41. Use of the enzyme component system according to Claims 1 to 40 in a process for the enzymatic printing ink removal during the deinking of waste paper, whereby a reducing agent such as sodium bisulfite, sodium dithionite, ascorbic acid, a thiol compound, mercapto compound or glutathione, but preferably sodium bisulfite and/or sodium dithionite, is added at a concentration from 0.1 to 1000 mg per kg of air-dried waste paper and preferably at a concentration from 0.1 to 200 mg per kg of waste paper.

42. Use of the enzyme component system according to Claims 1 to 41 in a process for the enzymatic printing ink removal during the deinking of waste paper, whereby, to collect the printing ink particles and to produce foam during flotation, a commercial collector, preferably of the Incopur brand, for example Incopur RSGA, is used at a concentration from 1 to 5000 mg per kg of air-dried waste paper and preferably from 1 to 1000 mg per kg of waste paper.

43. Use of the enzyme component system according to Claims 1 to 42 in a process for the enzymatic printing ink removal during the deinking of waste paper, whereby additional enzymes such as cellulases and/or hemicellulases such as xylanase and/or mannanase and/or pectinases and/or oxidoreductases are added.

44. Use of the enzyme component system according to Claims 1 to 43 as an oxidation system in organic synthesis, whereby the reaction of the enzyme component system is carried out at pH 2 to 11, preferably pH 3 to 6, at a temperature from 20 to 95 °C, preferably from 40 to 95 °C, in the presence of oxygen or air at atmospheric pressure or slightly positive O<sub>2</sub> pressure (up to 2 bar), and system component 1, namely lipase from *Humicola lanuginosa* is used at a concentration from 0.05 to 5 mg, preferably from 0.05 to 3 mg, and system component 2, namely one or more fatty acids, preferably C<sub>8</sub> to C<sub>18</sub> fatty acids, preferably tetradecanoic acid and/or dodecanoic acid, is used at a concentration from 0.05 to 100 mg, preferably at a concentration from 0.05 to 30 mg, and system component 3, namely the oxidant precursor, preferably H<sub>2</sub>O<sub>2</sub>, is used at a concentration from 0.05 to 100 mg (100%), preferably at a concentration from 0.05 to 30 mg, and system component 4, namely a ketone, preferably a benzophenone, is used at a concentration from 0.05 to 100 mg, preferably at a concentration from 0.05 to 30 mg, the concentrations in all cases being based on 10 mmols of substrate.

45. Use of the enzyme component system according to Claims 1 to 44 as an oxidation system in organic synthesis, whereby, for example, an aromatic alcohol or an aromatic methyl compound is used as the substrate for the oxidation reaction according to the invention.

46. Use of the enzyme component system according to Claims 1 to 45 in a process for the enzymatic liquefaction of coal, whereby the reaction of the enzyme component system is carried out at a pH from 2 to 11, preferably at pH 3 to 9, at a temperature from 20 to 95 °C, preferably from 40 to 95 °C, at a coal slurry consistency from 0.5 to 40%, preferably from 4 to 15%, in the presence

of oxygen or air at atmospheric pressure or a slightly positive  $O_2$  pressure (up to 2 bar), and system component 1, namely lipase from *Humicola lanuginosa* is used at a concentration from 0.05 to 20 mg, preferably from 0.05 to 10 mg, and system component 2, namely one or more fatty acids, preferably  $C_8$  to  $C_{16}$  fatty acids, preferably tetradecanoic and/or dodecanoic acid, is used at a concentration from 0.05 to 100 mg, preferably from 0.05 to 50 mg, and system component 3, namely the oxidant precursor, preferably  $H_2O_2$ , is used at a concentration from 0.05 to 50 mg (100%), preferably from 0.05 to 50 mg, and system component 4, namely a ketone, preferably a benzophenone, is used at a concentration from 0.05 to 100 mg, preferably at a concentration from 0.05 to 50 mg, in each case based on 1 g of coal (lignite).

47. Use of the enzyme component system according to Claims 1 to 46 in a process for detergent bleaching, whereby the reaction of the enzyme component system is carried out at a pH from 2 to 12, preferably at pH 3 to 10, at a temperature from 20 to 95 °C, preferably from 30 to 95 °C, in the presence of oxygen or air at atmospheric pressure or at a slightly positive  $O_2$  pressure (up to 2 bar), and system component 1, namely lipase from *Humicola lanuginosa*, is used at a concentration from 0.05 to 20 mg, preferably from 0.05 to 10 mg, and system component 2, namely one or more fatty acids, preferably  $C_8$  to  $C_{16}$  fatty acids, preferably tetradecanoic and/or dodecanoic acid, is used at a concentration from 0.05 to 50 mg, preferably at a concentration from 0.05 to 20 mg, and system component 3, namely the oxidant precursor, preferably  $H_2O_2$ , is used at a concentration from 0.05 to 50 mg (100%), preferably at a concentration from 0.05 to 20 mg, and system component 4, namely a ketone, preferably a benzophenone, is used at a concentration from 0.05 to 50 mg, preferably at a concentration from 0.05 to 20 mg, in each case based on 100 mL of washing solution.

48. Use of the enzyme component system according to Claims 1 to 47 in a process for detergent bleaching, whereby the system is added to a detergent formulation with all its technically common and known deterative substances or detergent additives.

49. Use of the enzyme component system according to Claims 1 to 48 in a process for bleaching and/or decolorizing textile fabrics, whereby the reaction of the enzyme component system is carried out at a pH from 2 to 11, preferably at pH 3 to 9, at a temperature from 20 to 95 °C, preferably from 40 to 95 °C, at a fabric density from 0.5 to 40% and preferably from 4 to 15%, in the presence of oxygen or air at atmospheric pressure or at a slightly positive  $O_2$  pressure (up to 2 bar), and system component 1, namely lipase from *Humicola lanuginosa* is used at a concentration from 0.05 to 10 mg, preferably from 0.05 to 5 mg, and system component 2, namely one or more fatty acids, preferably  $C_8$  to  $C_{16}$  fatty acids, preferably tetradecanoic and/or dodecanoic acid, is used at a concentration from 0.05 to 20 mg, preferably from 0.05 to 10 mg, and system component 3, namely the oxidant precursor, preferably  $H_2O_2$ , is used at a concentration from 0.05 to 20 mg (100%), preferably at a concentration from 0.05 to 10 mg, and system component 4, namely a ketone, preferably a benzophenone, is used at a concentration from 0.05 to 20 mg, preferably at a concentration from 0.05 to 10 mg, in each case based on 1 g of denim.

# Declaration and Power of Attorney for Patent Application

## Erklärung für Patentanmeldungen mit Vollmacht

### German Language Declaration

Als nachstehend benannter Erfinder erkläre ich hiermit an Eides Statt:

daß mein Wohnsitz, meine Postanschrift und meine Staatsangehörigkeit den im nachstehenden nach meinem Namen aufgeführten Angaben entsprechen, daß ich nach bestem Wissen der ursprüngliche, erste und alleinige Erfinder (falls nachstehend nur ein Name angegeben ist) oder ein ursprünglicher, erster und Mitfinder (falls nachstehend mehrere Namen aufgeführt sind) des Gegenstandes bin, für den dieser Antrag gestellt wird und für den ein Patent für die Erfindung mit folgendem Titel beantragt wird:

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As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated next  
to my name.

I believe I am the original, first and sole inventor (if only one name  
is listed below) or an original, first and joint inventor (if plural  
names are listed below) of the subject matter which is claimed and  
for which a patent is sought on the invention entitled

Oxidation and bleaching system with enzyme-  
tically produced oxidizing agents

the specification of which is attached hereto unless the following  
box is checked:

was filed on JUNE 19, 1998  
as United States Application Number or PCT International  
Application Number PCT/DE98/01689  
\_\_\_\_\_ was amended on \_\_\_\_\_  
(if applicable).

I hereby state that I have reviewed and understand the contents of  
the above identified specification, including the claims, as amended  
by any amendment referred to above.

I acknowledge the duty to disclose information which is material to  
patentability as defined in Title 37, Code of Federal Regulations, §  
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## German Language Declaration

Ich beanspruche hiermit ausländische Prioritätsvorteile gemäß Title 35, US-Code, § 119 (a)-(d), bzw. § 365(b) aller unten aufgeführten Auslandsanmeldungen für Patente oder Erfindungsurkunden, oder § 365(c) aller PCT internationalen Anmeldungen, welche wenigstens ein Land außer den Vereinigten Staaten von Amerika betreffen, und habe nachstehend durch ankrenzende spezifische Auslandsanmeldungen für Patente bzw. Erfindungsurkunden oder PCT internationale Anmeldungen angegeben, deren Anzeigung dem der Anmeldung, für welche Priorität beansprucht wird, vorangeht.

Prior Foreign Applications  
(Frühere ausländische Anmeldungen)

(Number) (Nummer)	(Country) (Land)
197 26 323.2	Germany
(Number) (Nummer)	(Country) (Land)

Ich beanspruche hiermit Prioritätsvorteile unter Title 35, US-Code, § 119(c) aller US-Hilfsanmeldungen wie unten aufgeführt:

(Application No.) (Ankennzeichen)	(Filing Date) (Anmeldetag)
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(Application No.) (Ankennzeichen)	(Filing Date) (Anmeldetag)
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Priority Not Claimed  
Priorität nicht beansprucht

(Day/Month/Year Filed) (Tag/Monat/Jahr der Anmeldung)
20. June 1997
(Day/Month/Year Filed) (Tag/Monat/Jahr der Anmeldung)

I hereby claim the benefit under Title 35, United States Code, § 119(c) of any United States provisional application(s) listed below:

I hereby claim the benefit under Title 35, United States Code, § 120 of any United States application(s), or § 365(c) of any PCT international application designating the United States, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT international application in the manner provided by the first paragraph of Title 35, United States Code, § 112, I acknowledge my duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations, § 1.56 which became available between the filing date of the prior application and the national or PCT international filing date of this application.

(Status) (patented, pending, abandoned)
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I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

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## German Language Declaration

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POWER OF ATTORNEY: As a named inventor, I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and transact all business in the Patent and Trademark Office connected therewith: (list name and registration number)  
 Dalbert W. Shultz No. 18,174; Francis M. Fluckey No. 21,138;  
 Karl S. Sawyer, Jr., No. 28,902; Chad D. Tillman No. 38,634;  
 Nancy Talavera Wood No. 38,334; Jack D. Todd No. P44,375;  
 Michael A. Tobin No. P43,956; Susan S. Jackson No. 41,302

Karl S. Sawyer, Jr.  
 Kennedy Covington Lobdell & Hickman, LLP  
 Bank of America Corporate Center, Suite 4200, 100 N.  
 Tryon Street, Charlotte, NC 28222-4006  
 Send Correspondence to:

Postanschrift:

Telefonische Anschrift: (Name und Telefonnummer)

Karl S. Sawyer, Jr. (704) 331-7400  
 Direct Telephone Calls to: (name and telephone number)

Vor- und Zuname des einzigen oder ersten Erfinders	1-00	Full name of sole or first inventor	Hans-Peter Call
Unterschrift des Erfinders	Datum	Inventor's signature	Date
Wohnsitz		Residence	59821 Amberg II, Germany
Staatsangehörigkeit		Citizenship	Germany
Postanschrift		Post Office Address	Kurfürstenstraße 6a, Amberg II, Germany
Vor- und Zuname des zweiten Mitfinders (falls zutreffend)		Full name of second joint inventor, if any	
Unterschrift des zweiten Erfinders	Datum	Second inventor's signature	Date
Wohnsitz		Residence	
Staatsangehörigkeit		Citizenship	
Postanschrift		Post Office Address	

(Im Falle dritter und weiterer Mitfinders sind die entsprechenden Informationen und Unterschriften hinzuzufügen.)

(Supply similar information and signature for third and subsequent joint inventors.)